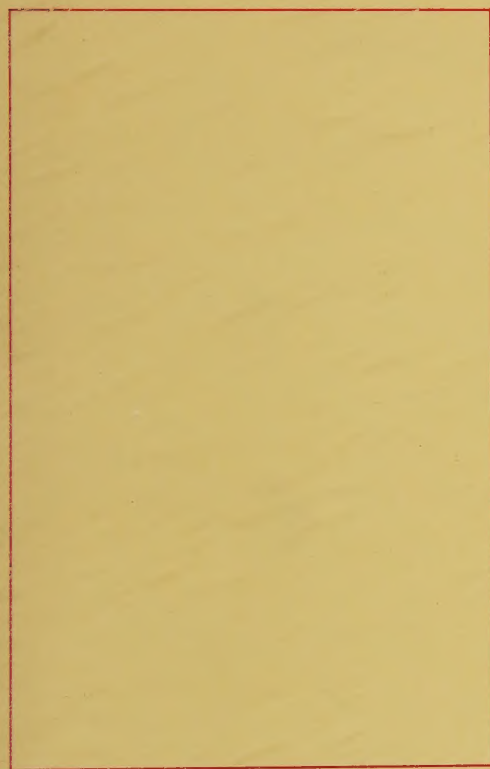


Lars Hernqvist

Lund 1984

POLYMORPHISM OF FATS





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Lars Hernqvist

1984



*There's more to the picture
than meets the eye*
Neil Young

PREFACE

Kåre - I just wanna say - *you're so good.*

Anita - You did a *tremendous* job.

Sune - You did a *really good* job.

Thanks also to Dan.

Thomas - Thank you for bringing the radio.

Tommy - Thank you for preserving the disorder at the phone desk.

Bodil - Thank you for doing the cover design.

Lars Seiron - Thank you for always having time.

Thank you also to everybody who helped me.

PUBLICATIONS

This thesis is based on the following papers, which will be referred to by Roman numerals in the text:

- I On the structure of triglycerides in the liquid state and fat crystallization.
L. Hernqvist
Fette-Seifen-Anstrichmittel, accepted for publication.
- II On the crystal structure of the β' -form of triglycerides and structural changes at the phase transitions $\text{liq.} \rightarrow \alpha \rightarrow \beta' \rightarrow \beta$.
L. Hernqvist and K. Larsson
Fette-Seifen-Anstrichmittel **84** 349-354 (1982).
- III Polymorphism of interesterified triglycerides and triglyceride mixtures.
L. Hernqvist, B. Herslöf and M. Herslöf
Fette-Seifen-Anstrichmittel, accepted for publication.
- IV On structural relations between lipid mesophases and isotropic reversed micellar (L2) solutions.
K. Fontell, L. Hernqvist, K. Larsson and J. Sjöblom
J. Colloid Interface Sci. **93** 453-460 (1983).
- V Polymorphism of rapeseed oil with a low content of erucic acid and possibilities to stabilize the β' -crystal form in fats.
L. Hernqvist, B. Herslöf, K. Larsson and O. Podlaha
J. Sci. Food Agric. **32** 1197-1202 (1981).
- VI Diglycerides as a stabilizer of the β' -crystal form in margarine and fats.
L. Hernqvist and K. Anjou
Fette-Seifen-Anstrichmittel **85** 64-66 (1983).

CONTENTS

Summary	11
Sammanfattning	12
Introduction	13
Polymorphism of triglycerides	14
Techniques	14
The structure of triglycerides	16
The chain packing of hydrocarbons	16
The hexagonal - H subcell	16
The orthorhombic - $O\perp$ subcell	16
The triclinic - T// subcell	18
The chain orientation	18
The liquid state	20
The α -form	22
The β' -form	24
The β -form	31
The structure of complex triglycerides	35
Polymorphic transitions of triglycerides.....	40
The polymorphic behaviour of complex triglycerides	43
Technical applications	43
Margarine.....	43
Chocolate	47
Future applications.....	49
References	50

SUMMARY

The polymorphism of triglycerides has been studied with special regard to technical applications. The techniques used were X-ray diffraction, Raman spectroscopy and differential scanning calorimetry. The chain disorder of triglyceride molecules in the liquid state was found to be almost constant above the melting point. Similarities between triglycerides in the liquid state and the L2-phase of liquid-crystalline lipid phases were observed. The L2-phase is relevant in the production of margarine emulsion. In the α -form the chains are proposed to be disordered near the methyl end groups, and due to this disorder the structure is closely related to the lamellar liquid-crystalline phase. The main features of the structure of the β' -form of triundecanoin were derived. The structure within the molecular layer is quite similar to that of the β -form. A second β' -form, β'_2 , of triundecanoin has been observed and it differs from the β'_1 -form in the orientation of the chain packing subcell $0\perp$ in relation to the true unit cell.

In order to understand the relations between the polymorphism of simple triglycerides and natural fats, the polymorphic behaviour of complex systems consisting of well defined triglycerides, produced by using interesterification of simple triglycerides, has also been studied.

It was found that diglycerides can be used to reduce the rapid $\beta' \rightarrow \beta$ transition speed occurring in some fats (*e.g.* hydrogenated rapeseed oil with low content of erucic acid). These results were evaluated for margarine production in pilot-plant scale.

SAMMANFATTNING

Triglyceridernas polymorfi har studerats med hjälp av röntgendiffraktion, Raman spektroskopi och differentiell svepkalorimetri.

Studium av triglycerider i smält tillstånd visar att graden av oordning i kolvätekedjorna är i det närmaste konstant över stora temperaturintervall. Likheten mellan triglyceridsmältan och L2-fasen av flytande kristallina lipidfaser har också iakttagits. L2-fasen bildas förmodligen vid tillverkning av margarinemulsioner.

Kolvätekedjorna föreslås vara oordnade nära metylgapet då α -formen föreligger. Denna oordning gör att α -formen visar likheter med lamellära flytande kristallina faser av lipider. Huvuddragen av β' -formen av triundecanoin har bestämts. Strukturen visar stora likheter med β -formen. Två β' -former av triundecanoin har detekterats, skillnaden mellan de två formerna (β'_1 och β'_2) ligger i den ortorombiska subcellens orientering i förhållande till den verkliga enhetscellen.

Genom att omestra blandningar av enkla triglycerider har komplexa system erhållits. Dessa har därefter studerats vad gäller polymorfiska omvandlingar, för att utröna sambanden mellan enkla triglyceriders och naturliga fetters polymorfi.

Genom att använda diglycerider har den snabba $\beta' \rightarrow \beta$ omvandlingen, som t ex hydrerat rapsfett med låg erukasyrahalt uppvisar, kunnat reduceras. Detta har även bekräftats genom försök i pilot-plant skala.

INTRODUCTION

The functional properties of fats are very important in many food systems. The type of dispersion, the solid-liquid ratio and the occurring crystal form have often a strong influence on the behaviour of the entire system.

The triglyceride molecules in a fat can be packed in the solid state in alternative ways, each crystal form having different melting point. This phenomenon is called polymorphism. The existence of two melting points of tristearin was observed already 1849 (1). A third melting point was found 1853 by Duffy (2) followed by reports showing two or three melting points for simple triglycerides (3-11). The reason why simple triglycerides give more than one melting point was not explained until 1934, when Clarkson and Malkin (12) demonstrated that the phenomenon was due to the occurrence of different crystal forms.

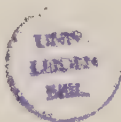
By relating the melting points to the X-ray diffraction pattern Malkin and coworkers came to the conclusion that simple triglycerides can be packed in four different ways. In 1950 Lutton and coworkers (13) reported the existence of three forms only. This started an intense debate between the groups of Lutton and Malkin (*cf.* Ref. (14)). The confusion did not end until new results, based on infrared spectroscopy studies, were presented by Chapman (*cf.* Ref. (15)), which supported the view of Lutton. In 1964 Larsson (16) reported new structural results on the different polymorphic forms, based on X-ray diffraction studies. An analysis of the accommodation of various types of triglyceride molecules into the most stable crystal form, the β -form, was later reported by de Jong (17).

The evolution of the present knowledge of the polymorphism in triglycerides can be studied in more detail in various articles (13-23).

Vand and Bell were the first to study a triglyceride by X-ray single-crystal methods (24). They analyzed the packing of the hydrocarbon chains in the β -form of trilaurin. This crystal structure determination, however, was not complete.

In 1963 the crystal structure of the β -form of the triglyceride of 11-bromoundecanoic acid, isomorphous with trilaurin, was determined by Larsson (25). The structure of the β -form of tricaprins was determined simultaneously by Jensen and Mabis (26). Up to this date no other crystal structure than the β -form is completely determined. Since all the different polymorphic forms of fats occur and influence the physical properties of various food systems, knowledge about the structure of triglycerides in the different crystal forms is important.

The present work deals with the polymorphism of triglycerides with special regard to technical applications.



POLYMORPHISM OF TRIGLYCERIDES

Techniques

The best technique to use for identification of the different polymorphic forms present in a fat system is X-ray diffraction. The three main polymorphic forms α , β' and β are defined according to the following criteria (19):

1. A form which gives only one strong short-spacing line near 4.15 Å is termed α .
2. A form showing two strong short-spacing lines near 4.20 and 3.80 Å or three strong lines near 4.27-3.97 and 3.71 Å, and which also exhibits a doublet in the 720 cm^{-1} region of the infrared absorption spectrum, is called β' .
3. A form which does not satisfy criteria 1 or 2 is called β .

When two or more crystal forms of a compound receive the same name they should be distinguished by subscripts, *e.g.* β_1 , β_2 and it is recommended that they are numbered in the order of decreasing melting points.

In the present work it was found useful to apply the term sub- α in order to show that the actual form is formed reversibly from the α -form at cooling.

By recording the diffraction pattern *versus* temperature (DPT-diagram) the phase transitions can be followed. The DPT-camera used in this work was constructed according to the principles described by Stenhagen (27), see Fig. 1.

Only X-ray single-crystal investigations can give total information on the crystal structures. Due to extreme difficulties in growing large single crystals, however, only the structure of the most stable form, the β -form, has been fully derived. A proposed structure of the β' -form of triundecanoin is given, which is based on uncomplete single-crystal data (II).

Many other techniques have been used to study the polymorphism of triglycerides (15), *e.g.* differential scanning calorimetry, nuclear magnetic resonance, infrared spectroscopy. The disadvantage, however, with most techniques is that the information obtained is rather limited and needs to be supported by other techniques. Differential scanning calorimetry, for instance, can give well-resolved peaks. Since only the net energy for the system is recorded, however, overlapping transitions can occur without being detected. Raman spectroscopy is another technique that also can be used in order to characterize lipids in the solid state (28,29).

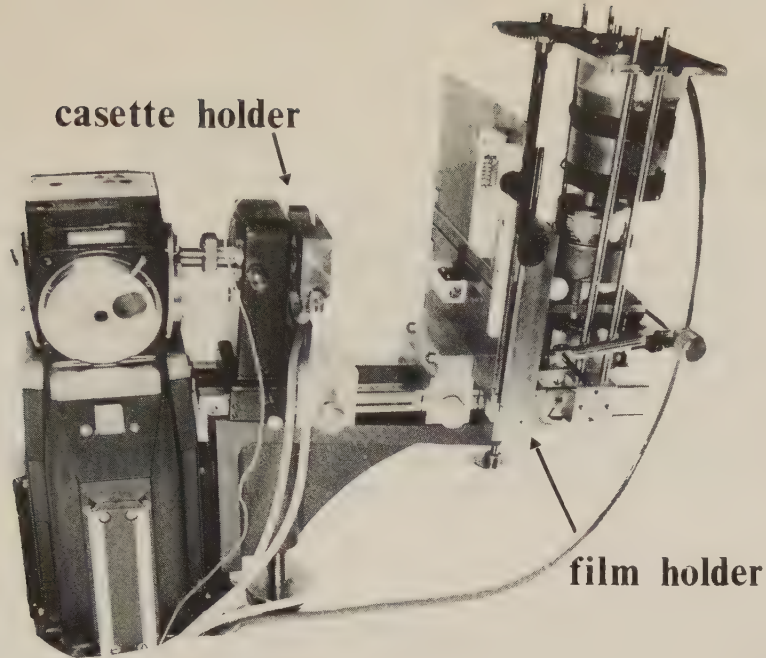
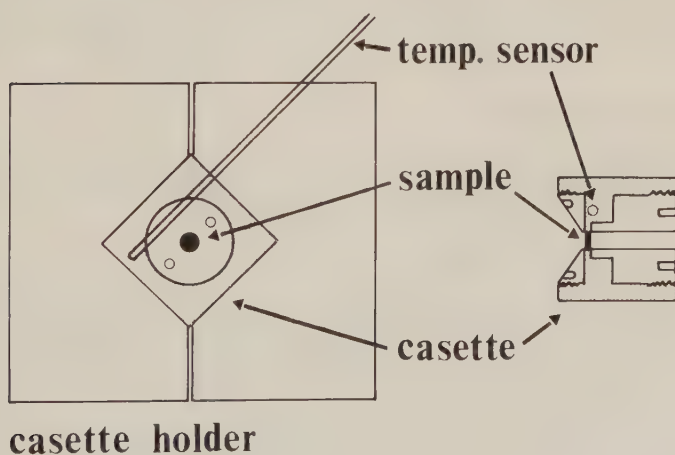


Fig. 1 DPT-camera (above) constructed for the present studies of fat polymorphism and lipid-water systems. A sample holder (below) requiring a few mg only utilizes mica window to avoid background scattering, and is also designed for microprocessor temperature control. The heat transfer was checked and found to be extremely efficient compared to conventional constructions. In the present set-up a circulating liquid is used for temperature control. The film, with planar geometry, moves synchronously with temperature, and the sample-to-film distance can be varied.



The structure of triglycerides

The chain packing of hydrocarbons

Regardless which kind of functional group that is present, all hydrocarbon chains in long chain compounds are packed in one of a few possible ways. The best method to describe the chain packing is to use the subcell corresponding to the smallest repetition unit within the chain layer. The subcell concept was developed by Vand in connection with his X-ray diffraction studies of soaps (30). Theoretical analyses of possible hydrocarbon chain packing modes in terms of subcells have also been given by Kitaigorodskii (31,32) and by Segerman (33). All subcells have been discussed thoroughly in a recent review by Abrahamsson and coworkers (34). Only the subcells occurring in triglycerides are discussed in this thesis.

The hexagonal-H subcell

In the α -form of triglycerides the hydrocarbon chains are arranged according to the hexagonal subcell (H). The dimensions of the H-subcell, corresponding to the 4.15 Å short-spacing, are :

$$a_s = 4.8 \text{ \AA} \text{ and } c_s = 2.5 \text{ \AA}, (\text{cf. Refs. (16,34,35)}).$$

The H-subcell is shown in Fig. 2. The packing is identical with that of close-packed cylindrical rods, which indicates rotational freedom of the hydrocarbon chains in the α -form. Due to the anchoring of the hydrocarbon chains in the glycerol region, it is obvious that the chains can only perform oscillational movements in triglycerides. The chain disorder is probably higher the longer the distance to the glycerol groups. The higher amount of chain disorder for the α -form compared with the β' and β -form, as detected with Raman spectroscopy, is discussed in paper II.

The amount of disorder in the hexagonal chain packing is also apparent from dilatometric (36), nuclear magnetic resonance (37) and dielectric (38) data.

The orthorhombic- $O\perp$ subcell

The $O\perp$ subcell is shown in Fig. 3. This chain packing occurs in the β' -form of triglycerides. The dimensions of the $O\perp$ subcell are (39):

$$a_s = 7.40 \text{ \AA}; b_s = 4.95 \text{ \AA}; c_s = 2.55 \text{ \AA}$$

Compared with the hexagonal subcell, the orthorhombic subcell is more close-packed. Therefore the hydrocarbon chains do not have the same high degree of disorder. Consequently the melting point of the β' -form of any triglyceride is higher compared with that of the α -form.

In the orthorhombic subcell, the plane through every chain is perpendicular to those of its nearest neighbours.

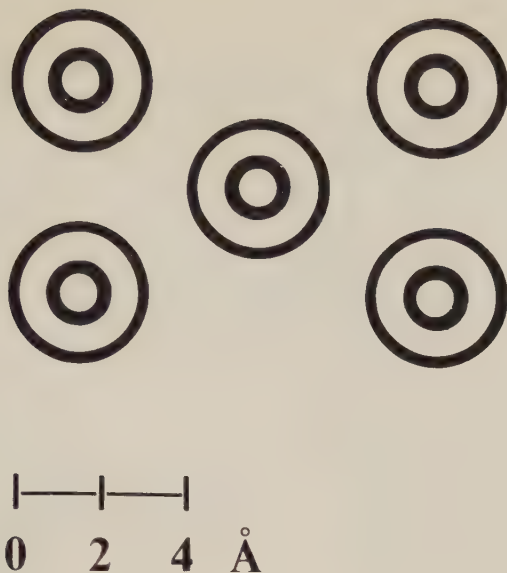


Fig. 2 The hexagonal subcell packing, H. The outer circles represent hydrogen positions and the inner ones carbon positions of rotating CH₂ groups.

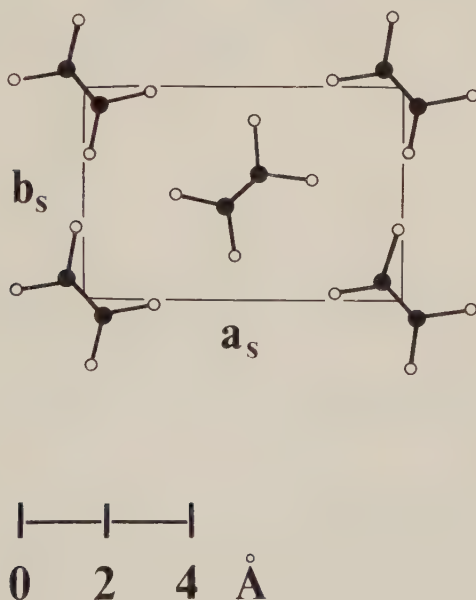


Fig. 3 The orthorhombic subcell packing, O1. One zig-zag period is seen in the direction of the hydrocarbon chains. Open circles are used for hydrogen atoms and filled circles for carbon atoms.

The triclinic-T// subcell

In the β -form of triglycerides the hydrocarbon chains are packed according to the triclinic-T// subcell. The dimensions of the T// -subcell can vary quite much, and in the case of trilaurin (24) they are:

$$a_s = 4.41 \text{ \AA}; b_s = 5.40 \text{ \AA}; c_s = 2.45 \text{ \AA} \\ \alpha_s = 74.8^\circ; \beta_s = 108.5^\circ; \gamma_s = 120.5^\circ$$

The triclinic subcell is shown in Fig. 4, and it can be seen that all chain planes are parallel. This is the best close packing of the three possible ways of close packing present in the case of triglycerides. Consequently the β -form shows the highest melting point.

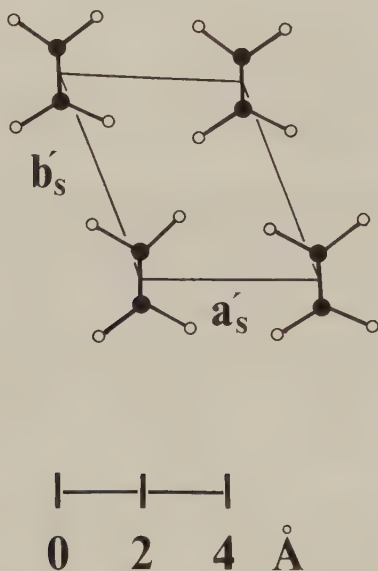


Fig. 4 The triclinic subcell packing, T//. One zig-zag period is seen in the direction of the hydrocarbon chains. Open circles are used for hydrogen atoms and filled circles for carbon atoms.

The chain orientation

Based on X-ray powder diffraction Clarkson and Malkin (12) proposed that two acyl chains in a triglyceride molecule are arranged adjacently and the third one points in the opposite direction. This principal structure of triglycerides is illustrated in Fig. 5a. The hydrocarbon chain can then be close packed according to the different subcells mentioned above but still be orientated according to the prin-

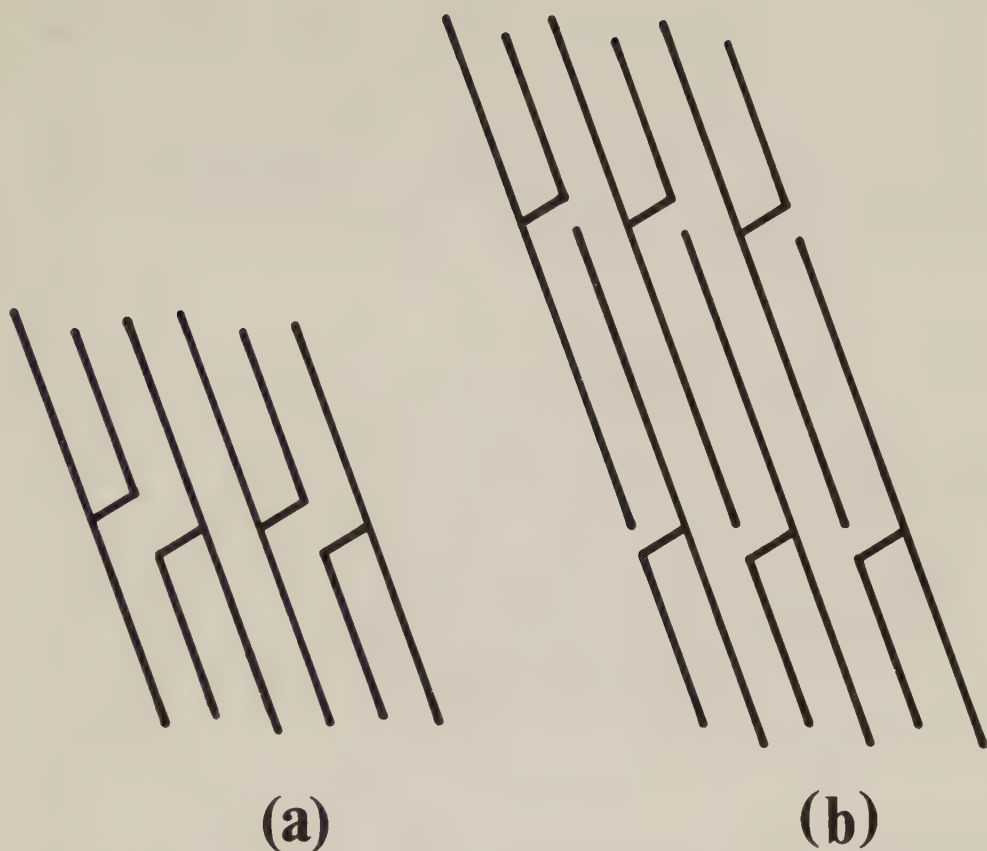


Fig. 5 Principal structure of triglycerides, double chain layer (a) and triple chain layer (b).

cipal packing in Fig. 5a. Even in the liquid form the triglyceride molecules seem to show a high order, *i.e.* the molecules are orientated roughly in the same way as in the different crystal forms. The difference being the long-range disorder and the high molecular mobility in the liquid state.

In 1948 Lutton (40) proposed an additional structure, *i.e.* triple chain layer. The structure is shown in Fig. 5b. This structure can occur when one chain in a triglyceride differs in length by four or more carbons, or if one chain is unsaturated, *i.e.* 1,3-distearoyl-2-oleoyl-glycerol.

The liquid state

Triglycerides in the liquid state are discussed in paper I. Based on X-ray technique and Raman spectroscopy, a structure of triglycerides in the liquid state is proposed, which is shown in Fig. 6. The hydrocarbon chains are disordered and the proportion of *gauche* conformation is large. As can be seen in the figure, the triglycerides are arranged tail to tail in the "tuning fork"-conformation. The proposed model is dynamic, *i.e.* both size and orientation of the lamellar units vary with diffusion rates of the molecules. When the temperature is increased, the size of the lamellar units decreases.

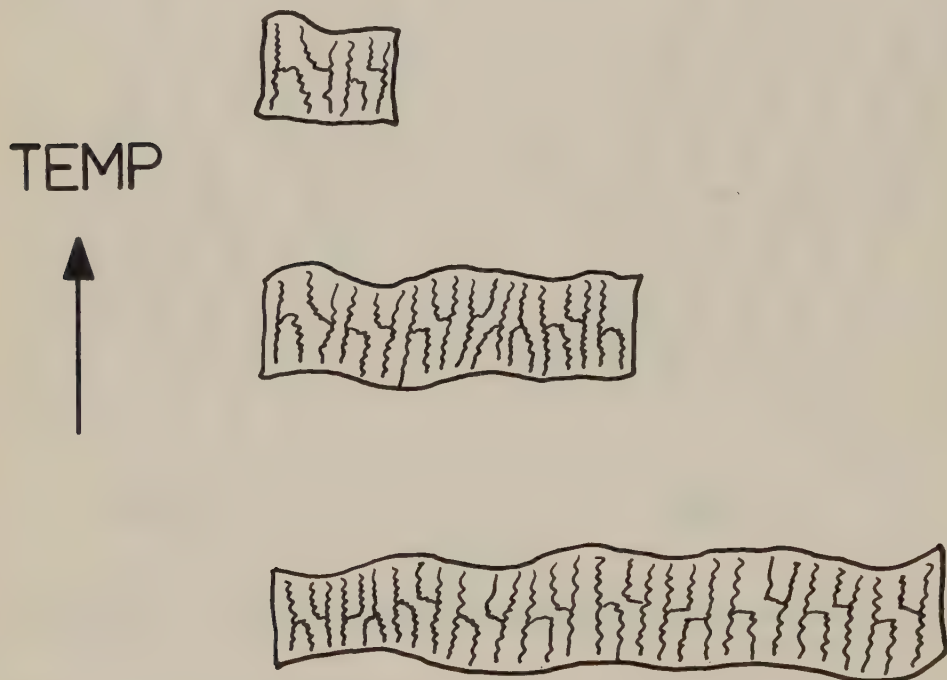


Fig. 6 Proposed structure of triglycerides in the liquid state.

The proposed reduction of the size of the lamellar units is based on line broadening, expressed as differences in half peak values for trimyristin, when the temperature is raised. The size of the lamellar units is hard to determine, an attempt was made by Larsson 1972 (22). By using Hosemann's paracrystal concept on line broadening, the approximate size just above the melting point was estimated to be about 200 Å.

There are reasons to believe that the size of the lamellae is the main thing shifting when the temperature is increased. Thus the long-spacing data from the X-ray scattering studies have been examined for trimyristin in the liquid state (I). The dominating intensity was interpreted as the bilayer thickness (22), and this value is constant from 45°C to 80°C.

The C-C stretching vibration region in the Raman spectra was also used to follow structural changes in the melt. Lipids with crystalline chains show two sharp peaks near 1065 cm⁻¹ and 1130 cm⁻¹, whereas these peaks are replaced towards a broad band near 1090 cm⁻¹ when the chains melt. The ratio 1080/1130 has been taken as a measure of the *trans/gauche* ratio (28), *i.e.* the degree of liquid type disorder. The ratio 1080/1130 is constant from 45°C to 80°C, *i.e.* the chain disorder seems to be constant.

Since the results of the X-ray scattering studies and the Raman spectroscopy studies are in good correlation, the degree of order in the liquid state is proposed to be almost constant when the temperature is increased. Furthermore, there is evidence indicating that the mobility of the hydrocarbon chains in the triglyceride molecule is increased with the distance to the glycerol group. The homologue series of simple triglycerides in the liquid state was studied both with X-ray technique and Raman spectroscopy (I). The average thickness of the bilayer of the homologue series shows that the larger the hydrocarbon chain, the smaller the contribution of the chain end to the d-value. The ratio 1080/1130 is drastically changed when the chain length is increased, *i.e.* the *gauche* proportion is much higher for the long carbon chain compared with a short one.

Liquid crystals of lipids show a similar behaviour. Due to the anchoring of one chain end at the lipid water interface, the *gauche* proportion (*i.e.* the chain disorder) is higher at the methyl end groups (41).

As a comparison, the bilayer thickness and the ratio 1080/1130, *i.e.* *gauche* proportion, were determined for 1-mono myristin and 1,2-dimyristin (I). Both 1-monomyristin and 1,2-dimyristin show higher value of the bilayer thickness (28.5 Å and 24.5 Å, respectively) compared with trimyristin (22 Å). This result is in good correlation with the difference in *gauche* proportion, which is highest for trimyristin and lowest for 1-monomyristin.

The half peak values (the line width of the half value of the intensity in relation to the background) for the melt of the different glycerides indicate that the structures of 1-mono- and 1,2-dimyristin are less disordered than the melt of trimyristin. This may be due to lateral molecular linking by hydrogen bonds, which cannot occur in the case of triglycerides.

When the temperature is decreased the lamellar units are increased in size until finally crystallization takes place. The probability of regions with neighbouring hydrocarbon chains orientated in all-*trans* also increases. Formation of a crystal nucleus can be regarded as the limiting point when formation of adjacent all-*trans* chains within the lamellar unit dominates over formation of *gauche* conformation (see Fig. 7).

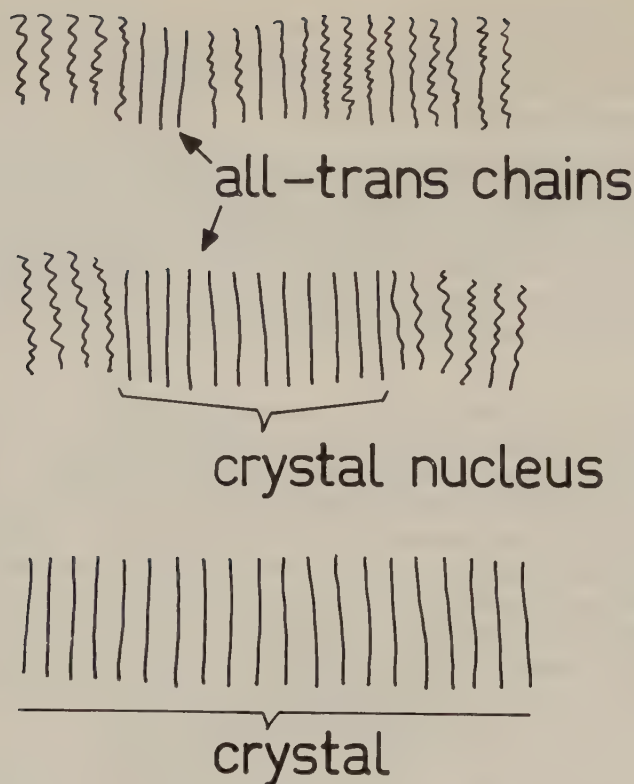


Fig. 7 Proposed mechanism of the crystallization of a triglyceride melt.

The α -form

When a triglyceride melt crystallizes, the α -form is normally the first form obtained. In Fig. 8 a proposed structure of the α -form of triglycerides is shown. The structure is projected along a short axis (around 5 Å). The main part of the hydrocarbon chains is considered to be oscillating and hexagonally close packed, with the methyl end group regions somewhat more disordered. This proposed structure of the α -form is based on X-ray diffraction and Raman spectroscopy studies (II). The dimension changes with temperature of the hexagonal H-subcell were studied with a DPT-camera (27). There is a minor shift in the 4.2 Å spacing of tristearin in the α -form (4.12 Å at 0°C and 4.18 Å at 50°C) and the bilayer thickness $d(001)$ was also found to decrease (51.6 Å at 0°C and 50.6 Å at 50°C). A reduction of this size of order of the long-spacing with temperature was the main reason for suggesting a liquid state of the hydrocarbon chains of liquid crystals by Luzzati *et al.* (42).

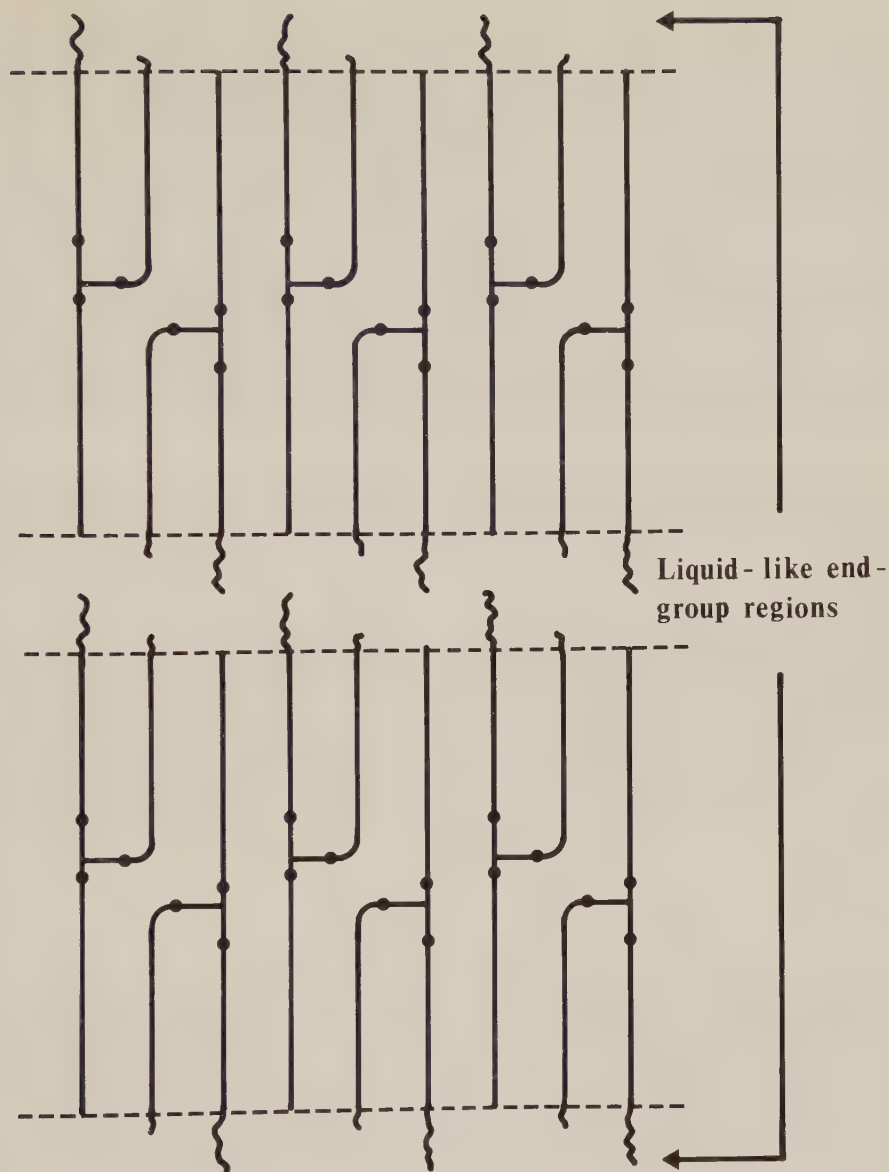


Fig. 8 Proposed structure of the α -form of triglycerides projected along a short axis. The chain axes are indicated; the main part of the hydrocarbon chains is oscillating and hexagonally close-packed; the methyl end group regions are somewhat more disordered, as in liquid crystals.

As mentioned above the ratio 1080/1130 in the Raman spectra is often taken as a measure of the *trans/gauche* ratio (28), *i.e.* the degree of liquid type disorder. The α -form shows a higher *trans/gauche* ratio compared with the β' - and β -forms of triglycerides, which indicates a higher chain disorder in the α -form (*cf.* Ref. 37). The results from the study of triglycerides in the liquid state (I), however, indicated that the methyl end group regions show a higher degree of chain disorder, compared with the parts of the chains near the glycerol residues. The α -form is proposed to show a similar kind of liquid-like end group regions as the chains of the lamellar liquid-crystal line phase of lipids. The physical properties of the α -form should therefore be expected to be related to those of the lamellar liquid crystals. The resistance against deformation parallel to the bilayer planes should thus be expected to be very low. Furthermore van den Tempel has reported that the α -form differs from ordinary crystals by not showing super-cooling, just like the lamellar form of liquid crystals (43).

Argument for a phase transition model, where the molecular packing is very similar for the three forms, α , β' and β , has been given (II). Thus the molecular dimeric units are the same with similar head-to-head packing of the glycerol groups.

If the long-spacing data of the different polymorphic forms are plotted against acyl chain length, the extrapolated intercept (corresponding to the glycerol end group region and the methyl gap) is much larger in the α -form compared with the other forms. This is consistent with the proposed structure of the α -form shown in Fig. 8. A serious disadvantage with the α -form from a molecular packing point of view is the irregular methyl end group region, as well as the hydrocarbon chain oscillation, and it is therefore natural that the α -form is quickly transformed to the β' -form.

The β' -form

The hydrocarbon chains in the β' -form of triglycerides are arranged according to the orthorhombic $0\perp$ -subcell. From a single-crystal study of triundecanoin the main structural features have been derived (II), as shown in Fig. 9. Since the detailed structure of the glycerol groups is not known, the glycerol group skeleton is indicated by thick bars. It can be seen that the methyl groups are packed in a better way compared to the situation in the α -form (see Fig. 8). The geometric relations between the (ab)-lattices in the β' - and β -forms are shown in Fig. 10. The positions of the chain axes and the space available indicate that the glycerol group region can have the same structure as in the β -form. As the orthorhombic chain packing requires an even number of chains laterally arranged in the unit cell (due to the fact that every hydrocarbon chain is perpendicular to its neighbours) there are six chains in each hydrocarbon chain layer of the unit cell. Thus, even if the glycerol group structure of the β' -form would be identical to that of the β -form, there must still be two molecules in the asymmetric unit. Beside this difference from the β -form (with all zig-zag planes parallel) the structure of the (010)-projection of the β -form and of the (010)-projection of the β' -form, shown in Fig. 9, are closely related.

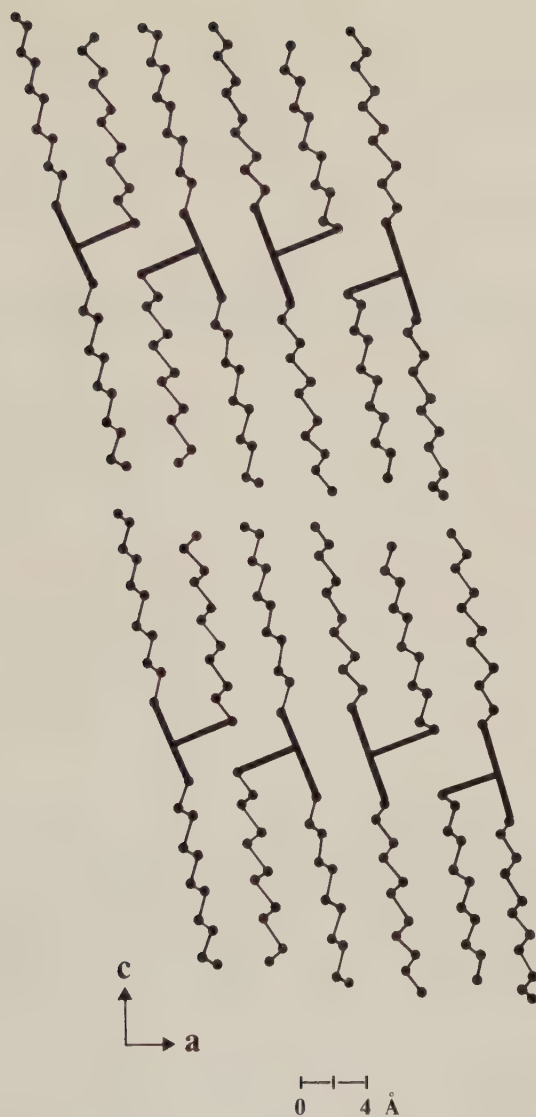


Fig. 9 Main features of the structure of the (010)-projection of the β' -form of triundecanoin; the glycerol group skeleton is indicated by thick bars.

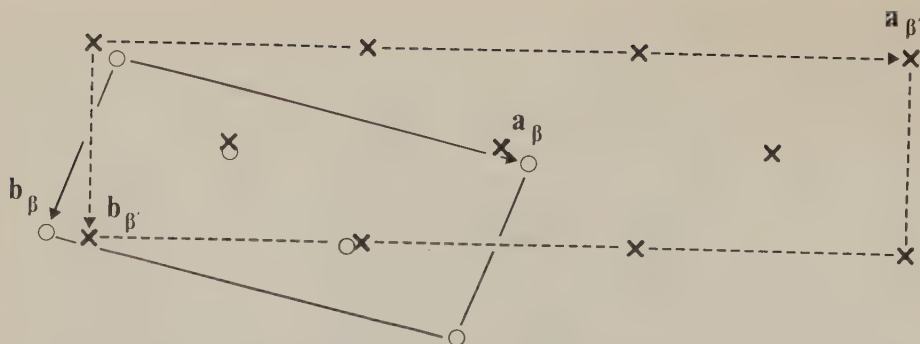


Fig. 10 Geometric relations between the (ab)-lattices in the β' - and β -forms, and the chain axes positions in the β -form (circles) and β' -form (crosses).

The (100)-projection, however, shows a structure quite different from that of the β -form. The chains are tilted in relation to the end-group planes, and from the space group symmetry, it follows that there are two chain tilt directions. The double-chain layers location between $z = -1/4$ and $z = +1/4$ are thus tilted in an opposite direction, compared to the double-chain layer located between $z = 1/4$ and $z = 3/4$. The question if the chain tilt alternates in the glycerol group region or at the methyl end groups cannot be unambiguously determined from these X-ray data. In an earlier discussion of the β' -form (16), the space group was proposed to be $P2_12_12_1$. The new single-crystal data, however, show that the earlier observed apparent orthorhombic symmetry was due to twinning. As the dimensions correspond to an orthorhombic unit cell, such twinning is strongly favoured. An earlier interpretation of alternation of chain tilt at the glycerol groups (16) was based on the space group $P2_12_12_1$. According to the present data, however, it is possible that molecular double-chain layer of the β' -form can have the same general structure as that of the β -form. Furthermore it is thus possible that the chain axes only need minor positional changes at the $\beta' \rightarrow \beta$ transition (Fig. 10). The angle of tilt is also very close in the two forms (the double-layer thicknesses for the β' - and β -forms of triundecanoin are 29.5 Å and 29.6 Å, respectively). On this basis it was proposed that the change of chain tilt takes place at the methyl end group plane (see Fig. 11).

A DPT-diagram of triundecanoin is shown in Fig. 12, and it can be seen that there are two β' -forms. These two forms should thus be classified as β'_2 and β'_1 (19). Consequently triundecanoin undergoes the $\alpha \rightarrow \beta'_2 \rightarrow \beta'_1$ transition before it finally melts at 30°C. The homologue series of odd simple triglycerides all show the existence of two β' -forms (II).

The short-spacing data for the two β' -forms of triundecanoin are:

$\beta'_2 = 3.91$ (m), 4.22 (s) and 4.36 (s); $\beta'_1 = 3.96$ (s), 4.08 (m), 4.32 (s) and 4.52 (w). The reason to this different intensity distribution of the two β' -forms can be explained in the following way. The dominating short-spacing lines from the β' -form correspond to the interplanar distances of the subcell $b_s/2$ ($= 3.8$ Å) and the



Fig. 11 Chain directions in the (100)-projection of the β' -form of triundecanoin indicated by one molecule in each layer of the unit cell.

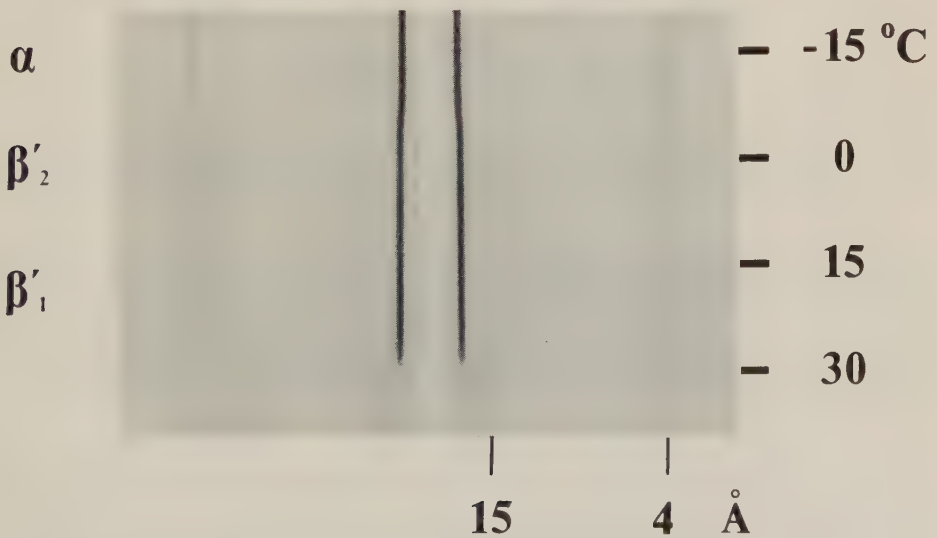


Fig. 12 DPT-diagram of the polymorphic transitions of triundecanoin when the α -form is heated ($0.4^{\circ}\text{C}/\text{min}$).

asbs-diagonal/2 ($= 4.2 \text{ \AA}$) (see Fig. 3). The orientation of the subcell within the true unit cell, however, will also influence the intensities of the observed short-spacing lines, and an example of one possible orientation is shown in Fig. 13. This figure illustrates the orientation of the chain packing subcell so that the subcell plane (011) is parallel to plane (001) of the true unit cell (this mutual orientation is usually described as $0\perp(011)$). With such an orientation, the interplanar spacing $b_s/2 = \sqrt{b_s^2 + c_s^2}/2 = 4.0 \text{ \AA}$ should give rise to a strong X-ray reflexion intensity. This subcell orientation will consequently be expected to exhibit three short-spacing lines, *i.e.* 3.8, 4.0 and 4.2 $\text{\AA}$. The β'_1 -form described above has the orientation of the $0\perp$ subcell defined by $0\perp(111)$.

This orientation should analogously be expected to give short-spacing lines at 4.0 $\text{\AA}$ ($=\sqrt{b_s^2 + c_s^2}/2$) and at 4.5 $\text{\AA}$ ($=\sqrt{a_s b_s^2 + c_s^2}/2$ with $a_s b_s$ = subcell diagonal), which also was observed. The orientation $0\perp(101)$ should be expected to give an additional line at 4.3 $\text{\AA}$, which is in agreement with the observed short-spacing of the β'_2 -form. It was therefore proposed that β'_2 exhibits the chain packing end group plane $0\perp(101)$. The angles of tilt of the chains differ only a few degrees between these two $0\perp$ chain arrangements (about 71°C and 74°C , respectively, which is consistent with the long-spacings 29.9 $\text{\AA}$ and 30.3 $\text{\AA}$, of the two β' -forms respectively).

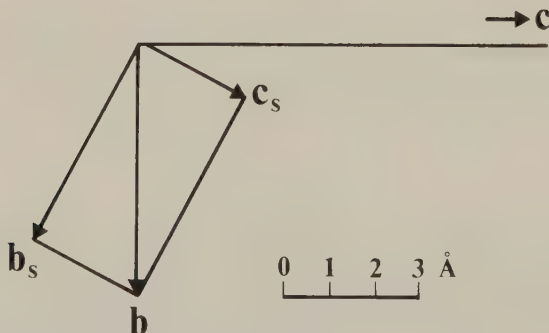


Fig. 13 A projection of unit cell along the a -axis and an orthorhombic chain packing subcell oriented with the $c_s b_s$ diagonal coinciding with the b -axis.

Some DSC-thermograms are shown in Fig. 14. The DSC used was a Perkin Elmer DSC-2C. All samples consist of triundecanoin (1.8 mg) heated at a scanning rate of $20^\circ\text{C}/\text{min}$, the difference between the three curves is how fast (5 , 10 or $40^\circ\text{C}/\text{min}$) the sample has been cooled before heating. Curve A ($5^\circ\text{C}/\text{min}$) gives a $\beta' \rightarrow \text{liq.}$ transition, curve B ($10^\circ\text{C}/\text{min}$) gives a $\beta'_2 \rightarrow \beta'_1 \rightarrow \text{liq.}$ transition and curve C ($40^\circ\text{C}/\text{min}$) gives a $\alpha \rightarrow \beta'_2 \rightarrow \beta'_1 \rightarrow \text{liq.}$ transition.

The crystallization temperature for triundecanoin varies when the cooling speed is shifted, *i.e.* $5^\circ\text{C}/\text{min}$ gives crystallization at 14°C ; $10^\circ\text{C}/\text{min}$, 9°C and $40^\circ\text{C}/\text{min}$, -4°C . Examination of the transition temperatures as studied by DPT (II) shows that $\alpha \rightarrow \beta'_2$ occurs at 5°C , $\beta'_2 \rightarrow \beta'_1$ at 11°C and $\beta'_1 \rightarrow \text{liq.}$ at 30°C . Consequently the DSC-data shown in Fig. 14 support the existence of the β'_2 -form.

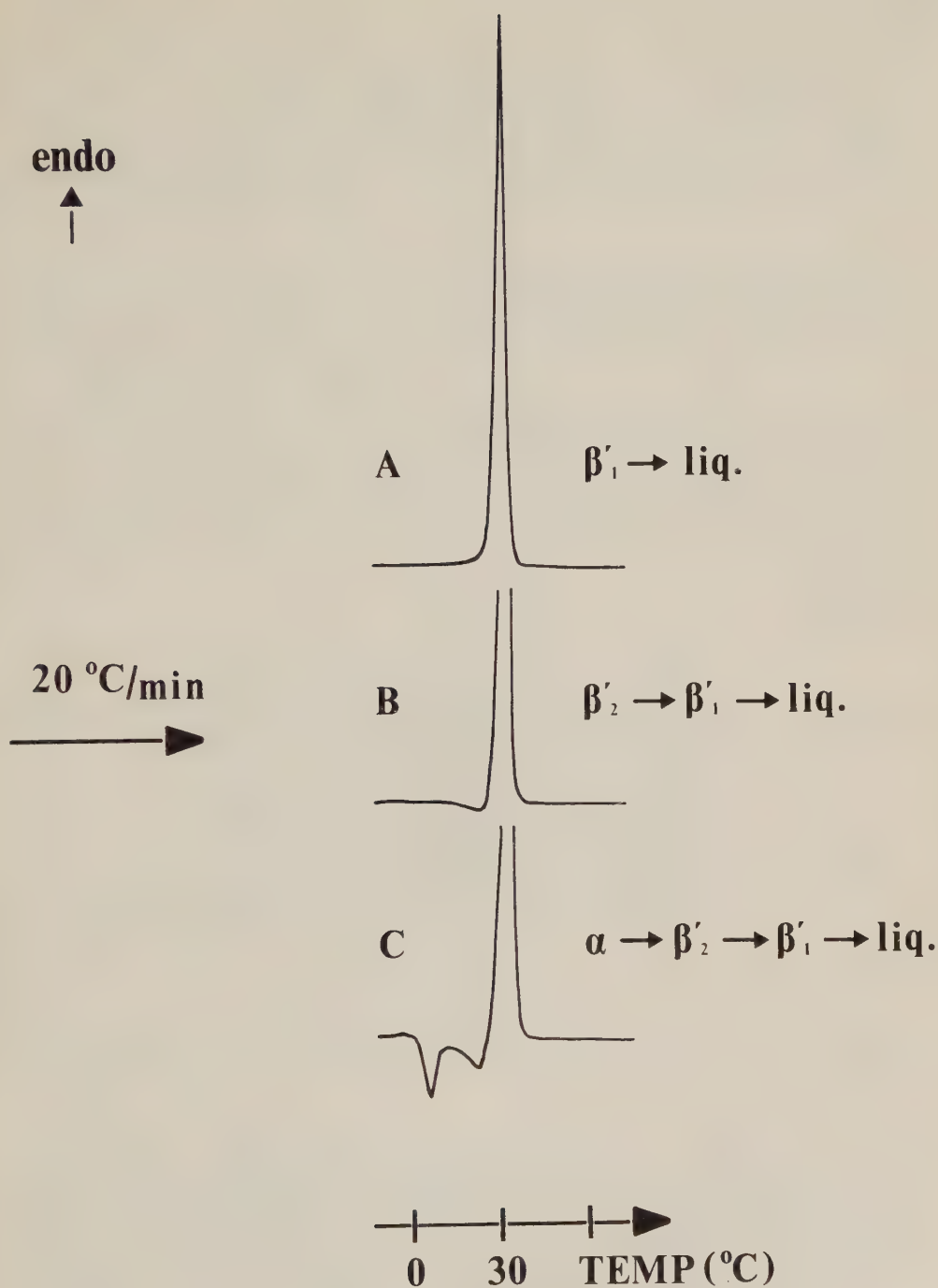


Fig. 14 DSC-thermograms of triundecanoin differently cooled (A, 5°C/min; B, 10°C/min and C, 40°C/min) prior to heating (20°C/min).

endo

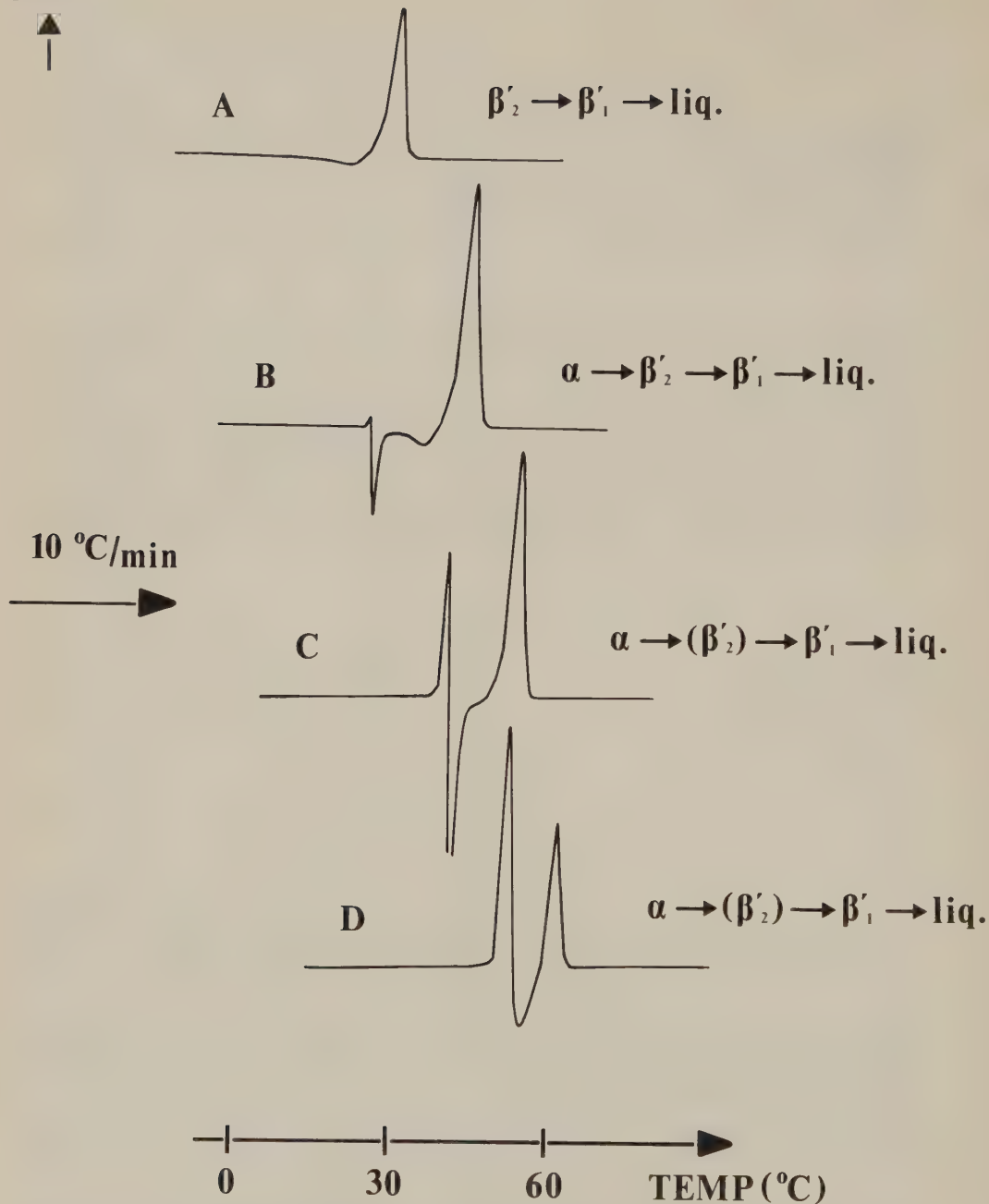


Fig. 15 DSC-thermograms of the homologue series of odd simple triglycerides (A - triundecanoin, B - tritridecanoin, C - tripentadecanoin and D - triheptadecanoin). All samples were cooled at a speed of 20°C/min prior to the thermograms were collected.

The homologue series of odd simple triglycerides have also been studied with DSC. The result is shown in Fig. 15. All samples were cooled at a speed of $20^{\circ}\text{C}/\text{min}$ prior to the thermograms were collected. These data are consistent with the data obtained from the DPT-studies discussed in paper II. The transitions indicated are based both on a comparison within the homologue series, and results also from DPT-studies. Hence the β'_2 is given in brackets. It probably exists before the β'_1 -form is obtained, but due to the overlapping of exo- and endothermic processes, it is impossible to see.

Two β' -forms have been reported for other triglycerides than odd simple triglycerides *i.e.* 2-stearo-dipalmitin (II) and tristearin (44). The short-spacing data given in the literature can, according to the arguments given above, be classified in terms of β'_1 and β'_2 .

If tribehenin is cooled down, the sub- α -form is developed (*cf.* Ref. (20)) (see DPT-diagram in Fig. 16). When the sub- α -form is obtained, an extra line occurs at $3.8\text{ \AA}$, which indicates that the hydrocarbon chains are packed according to the orthorhombic subcell. Consequently it can be referred to as a β' -form (19). However, since the transition is reversible (*cf.* Fig. 19), the name of sub- α is therefore preferred.

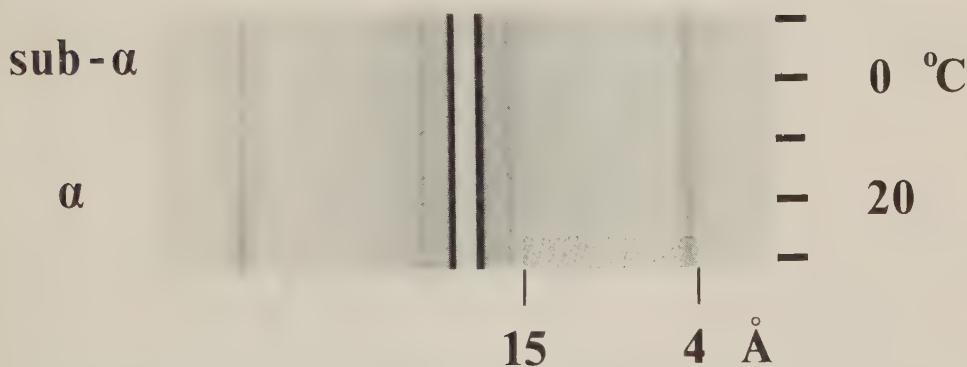


Fig. 16 DPT-diagram of tribehenin when the sub- α -form is heated ($0.4^{\circ}\text{C}/\text{min}$).

The β -form

In the β -form the hydrocarbon chains are arranged according to the triclinic T// subcell. The crystal structure of the β -form of trilaurin (25,26) is shown in Fig. 17. The chains are tilted in relation to the end-group planes in the (100)-projection as well as in the (011)-projection.

If the three different forms of simple triglycerides, α , β' and β finally are compared, the structural relations are quite close. The hydrocarbon chains in all forms are

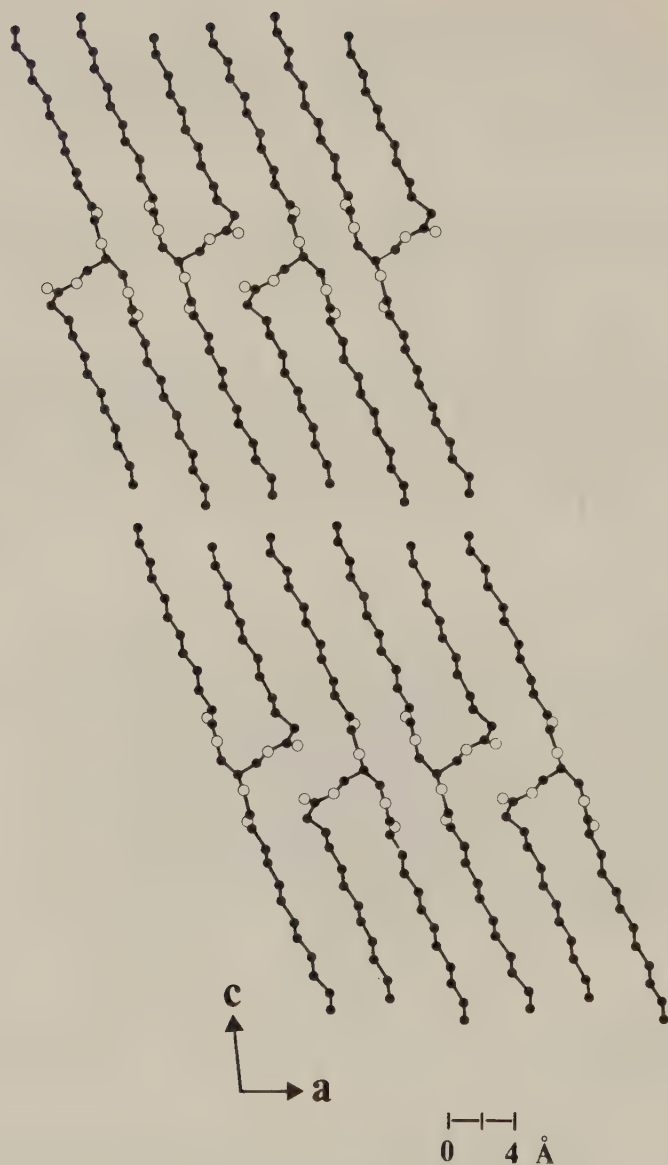
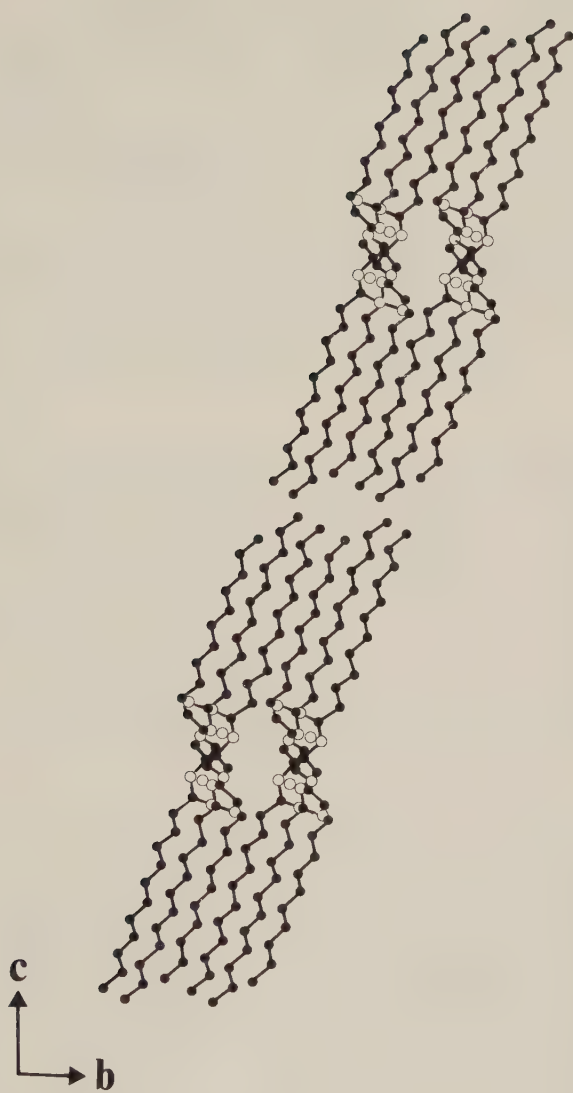


Fig. 17 Molecular arrangement in the β -form of trilaurin (25) as seen along the short unit cell axes.



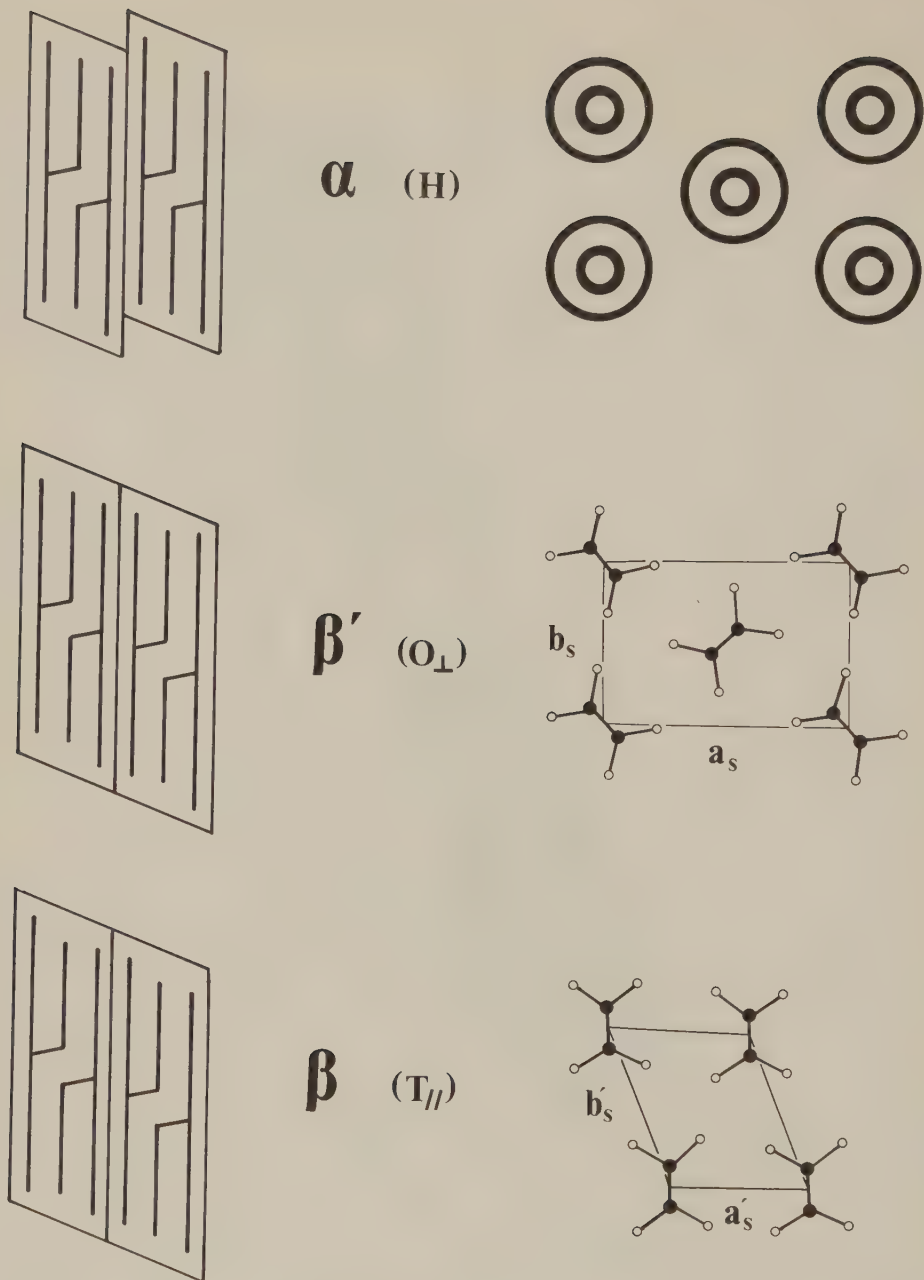


Fig. 18 Schematic orientation of the proposed triglyceride dimers of the α -, β' - and β -forms showed together with respective chain packing subcell.

arranged in the "tuning fork" form with the chains in 1- and 3-position pointing opposite to the chain in the 2-position. The molecular dimer can thus have the same structure in all three forms. The main difference is how the dimers are arranged in relation to each others, and in which way the hydrocarbons are close-packed. These proposed dimers are shown for the α , β' and β -forms of triglycerides in Fig. 18, together with corresponding subcells.

The structure of complex triglycerides

The structures discussed above are based on investigations of simple saturated triglycerides, and relations of the X-ray diffraction data indicate that these structures are valid also for most complex triglycerides as well as mixtures of triglycerides. In a natural fat both chain length and degree of unsaturation can vary, but nevertheless the same polymorphic forms are obtained, as can be seen from the short-spacing data.

So far only the double chain length type of packing has been discussed, but as mentioned earlier triglycerides can also be packed in a way that triple chain length units are obtained (see Fig. 5). This can occur in both triglycerides containing saturated and triglycerides containing unsaturated fatty acids. In the case of saturated triglycerides, one chain in the triglyceride molecule needs to differ in length by at least four carbons. de Jong (17) recently discussed the alternative chain length structures in the β -form. If one chain in a triglyceride is unsaturated, *i.e.* 2-oleo-distearin, triple chain length units can be obtained (22) with all the oleic acyl chains in one layer. 2-Decanoin-diolein was recently proposed to give a triple chain length structure (17) with the olein chains in the first and third positions in one layer.

A DPT-diagram of 1-oleo-distearin is shown in Fig. 19. Examination of the long-spacing reveals that this triglyceride must be packed according to the triplechain length concept. This is perhaps surprising, since the unsaturated chains should be expected to be segregated in one layer if a triple chain structure should exist. Hence the acyl chain in the 1-position in the triglyceride probably is orientated in the opposite direction to the others, contrary to the β -form of tridodecanoin, where the acyl chain in the 2-position is pointing in the opposite direction to the others (25).

Some interesterified triglyceride systems have also been studied (III). The theoretical molar proportions of the triglycerides present in the triolein-tristearin interesterified system are: tristearin 6.4, 2-oleo-distearin 9.6, rac-1-oleo-distearin 19.2, rac-1-stearo-diolein 28.8, 2-stearo-diolein 14.4 and triolein 21.6. The interesterification reaction was checked by means of high performance liquid chromatography, and found to be fully randomized. The DPT-diagram of a triolein-tristearin interesterified system is shown in Fig. 20. The long-spacing indicates the existence of a triple chain structure in the sub- α and α -forms which disappears when the $\alpha \rightarrow \beta'$ -transition occurs.

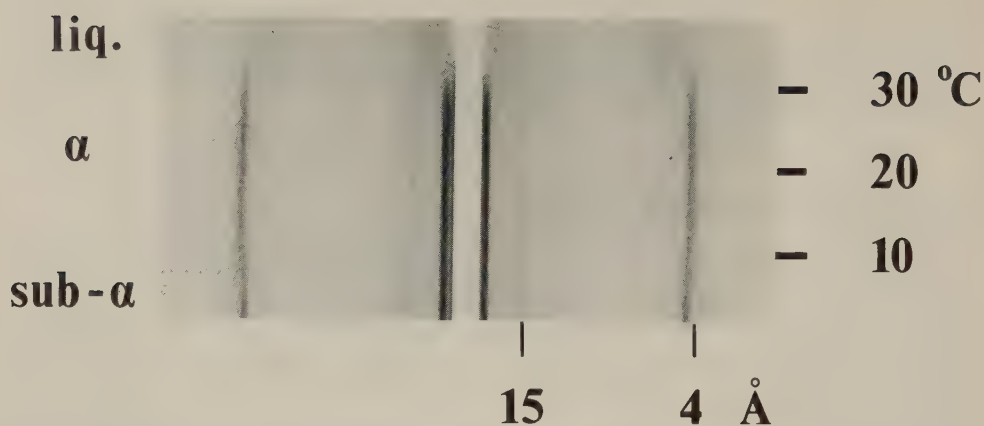


Fig. 19 DPT-diagram of the polymorphic transitions of 1-oleo-distearin when the melt is cooled (0.4°C/min).

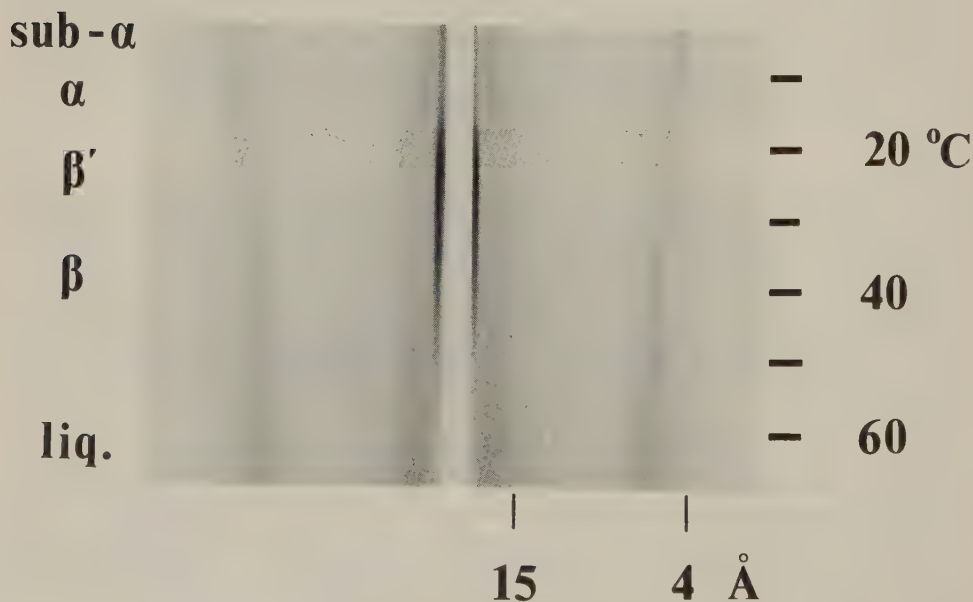


Fig. 20 DPT-diagram of the polymorphic transitions of an interesterified triolein-tristearin system (molar proportions 3:2; heating speed 0.4°C/min).

By blending 2-oleo-distearin, rac-1-oleo-distearin, tristearin and triolein in the molar proportions 9.6, 19.2, 6.4, 64.8, a system equal to the interesterified triolein-tristearin system is obtained, with the exception of rac-1-stearo-diolein and 2-stearo-diolein. This mixture, however, does not show triple chain length. Consequently the triple chain length order in the interesterified triolein-tristearin system is proposed to be due to the presence of 1-stearo-diolein and 2-stearo-diolein.

The 1-stearo-diolein and 2-stearo-diolein glycerides can either crystallize segregated from the rest of the triglycerides in a triple chain length arrangement or co-crystallize with the rest of the triglycerides present in the system. A proposed structure of these two triglycerides in the case of segregated crystallization is shown in Fig. 21. It should be pointed out that this segregation of the stearic acyl chains in one layer and the oleic acyl chains in two layers excludes the "normal" packing with the acyl chain in the 2-position pointing in the opposite direction (25) (*cf.* the triple chain length of 1-oleo-distearin discussed above).

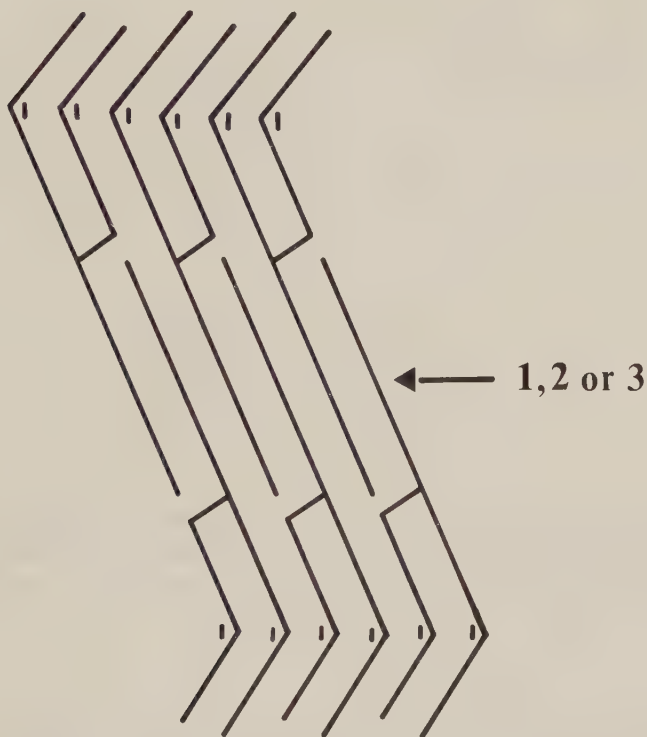


Fig. 21 Proposed structure of rac-1-stearo-diolein and 2-stearo-diolein arranged according to the triple chain layer concept. The oleic acyl chains are segregated in the outer layers and the stearic acyl chains, either in 1, 2 or 3 position, are packed in the middle.

If co-crystallization occurs, the oleic acyl chains are proposed to be packed in one layer and the stearic acyl chains in another layer together with other stearic acyl chains. This would give a triple chain order with saturated chains in two layers and unsaturated chains in the third one (see Fig. 22).

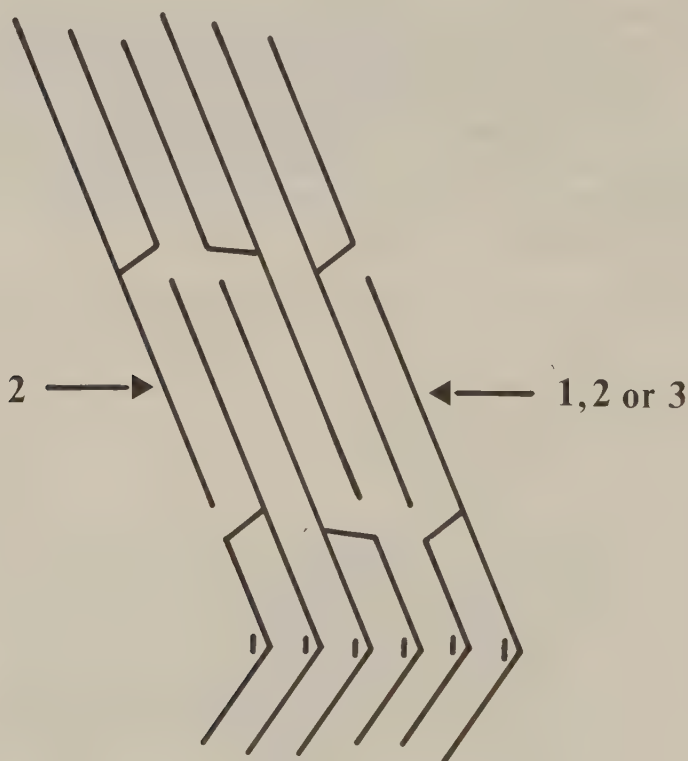


Fig. 22 Proposed structure of a co-crystallized mixture consisting of rac-1-stearo-diolein, 2-stearo-diolein and tristearin, arranged according to the triple chain length concept. All oleic acyl chains segregated in one layer.

When the triolein-tristearin interesterified system undergoes the $\alpha \rightarrow \beta'$ transition, the triple chain length order disappears (see Fig. 20). The $\alpha \rightarrow \beta'$ transition in Fig. 20 coincides with the melting of 2-stearo-diolein and 1-stearo-diolein, thus the molecular packing is rearranged giving double chain layer. This proposition is based on the fact that the α -crystal forms are partly disordered, the mobility of the hydrocarbon chains is quite high, hence giving space for irregularities in the chain packing.

The crystallization properties of triglycerides containing elaidic acyl chains were also studied (III). The polymorphic behaviour of interesterified systems of trielaidin-tristearin and that of triolein-tristearin were thus compared. The results indicate that the presence of elaidic acyl chains has a weak influence on the polymorphic behaviour, the main difference being the shift in all transition temperatures. Trielaidin and tristearin show the same polymorphic transitions on heating, the difference being the melting point. A binary mixture of trielaidin and tristearin shows separate crystallization of the components.

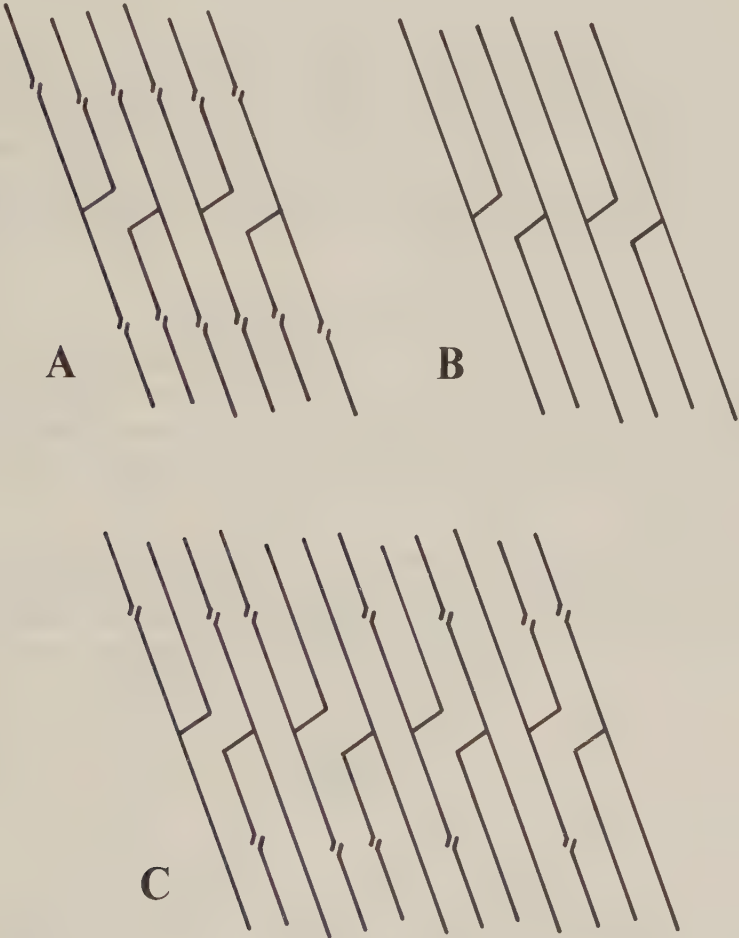


Fig. 23 The main characteristics of the β -forms of trielaidin (A), tristearin (B) and a mixture consisting of triglycerides containing both elaidic and stearic acyl chains (C).

The main characteristics of trielaidin, tristearin and a mixture consisting of triglycerides containing both elaidic and stearic acyl chains, all in the β -form, are shown in Fig. 23. The close relation between the structures is obvious. The *trans*-unsaturation in the elaidic acyl chain gives rise to a small distortion in the crystal packing, resulting in a lower melting point.

One conclusion from the discussions above is that the polymorphic behaviour of all triglycerides is very similar. Unsaturation and differences in chain length give defects in the packing of the hydrocarbon chains with lower melting temperatures as a consequence. *Trans*-unsaturation does not cause much disturbances in the chain packing (see Fig. 23), contrary to *cis*-unsaturation, as known earlier.

If the acyl chains have different chain lengths, this must give rise to a chain disorder in the methyl end group region. This higher disorder could influence the polymorphic transitions (*cf.* the polymorphic behaviour of tristearin (III) and 2-stearo-dipalmitin (II)). In some cases triple chain length occurs but in most complex systems double chain length is the most common. A critical factor in this connection is how fast the transition goes between the different polymorphic forms, which will be discussed below.

Polymorphic transitions of triglycerides

When the temperature of a triglyceride melt is decreased the lamellar units are proposed to increase in size (see Fig. 6). The probability of regions with neighbouring hydrocarbon chains orientated in all-*trans* also increases. When the formation of adjacent all-*trans* chains within a lamellar unit dominates over the formation of *gauche* conformation a crystal nucleus is obtained (see Fig. 7).

Crystallization can occur if the temperature is lower than the β -melting point. Usually triglycerides crystallize in the α -form, and then the $\alpha \rightarrow \beta' \rightarrow \beta$ transitions take place. This simple picture of the polymorphic transition is illustrated in Fig. 24. This illustration is not complete, however, which will be further considered below.

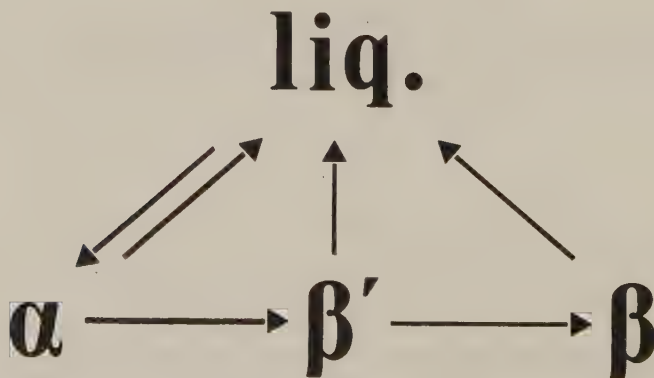


Fig. 24 Simple picture of the polymorphic transitions of triglycerides.

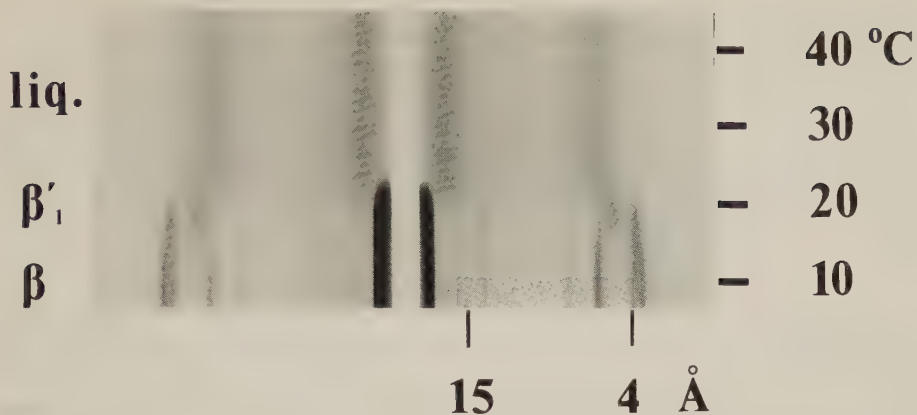


Fig. 25 DPT-diagram of the polymorphic transitions of trilaurin when the melt is cooled (0.4°C).

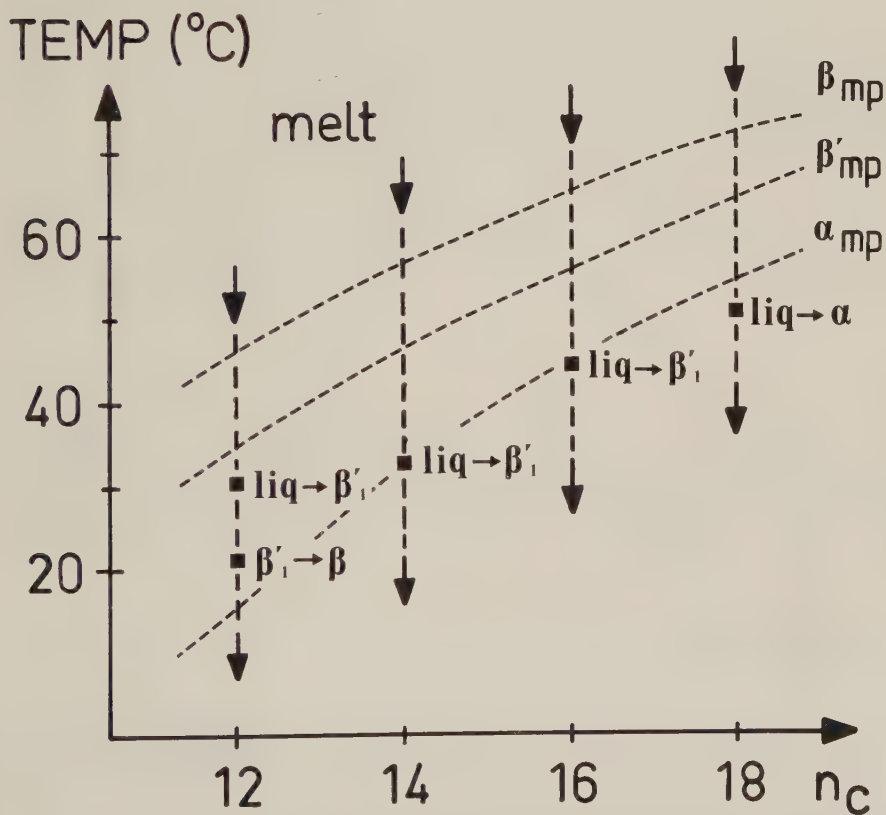


Fig. 26 Polymorphic transitions for the melt of simple triglycerides when slowly cooled ($0.4^\circ\text{C}/\text{min}$, studied with DPT).

The transition $\text{liq.} \rightarrow \beta'_1 \rightarrow \beta$ for trilaurin is shown in Fig. 25. When the crystallization temperatures of the trilaurin and tristearin are compared (see Fig. 26) the difference between the two triglycerides is quite pronounced. Tristearin crystallizes at the melting point of the α -form, while trilaurin crystallizes 15°C above its α -melting point. The reason why tristearin crystallizes at a comparatively low temperature might be due to the lower probability that a longer chain develops all-*trans*-conformation. By holding a tristearin melt isothermally above the α -melting point, *e.g.* at 60°C , the β -form is developed directly within about 40 minutes.

The cooling speed for the same triglyceride also influences the polymorphic behaviour. Effect of different cooling speed on the crystallization of triundecanoin, as studied by DSC, is shown in Fig. 14. The slow cooling ($5^\circ\text{C}/\text{min}$) gives the β' -form at 14°C , the medium-rate cooling ($10^\circ\text{C}/\text{min}$) gives the β'_2 -form at 9°C , and the fast cooling ($40^\circ\text{C}/\text{min}$) gives the α -form at -4°C .

If the data given above are considered, a more complex picture of the polymorphic transitions of triglycerides is obtained, which is illustrated in Fig. 27. All polymorphic forms can be obtained directly from the liquid state, except the sub- α -form. The transitions between different polymorphic forms are irreversible with the exception of the $\alpha \rightarrow \text{sub-}\alpha$ transition. The transition $\alpha \rightarrow \beta'_2 \rightarrow \beta'_1 \rightarrow \beta$ is the complete way for triglycerides to find the optimum packing of the molecules.

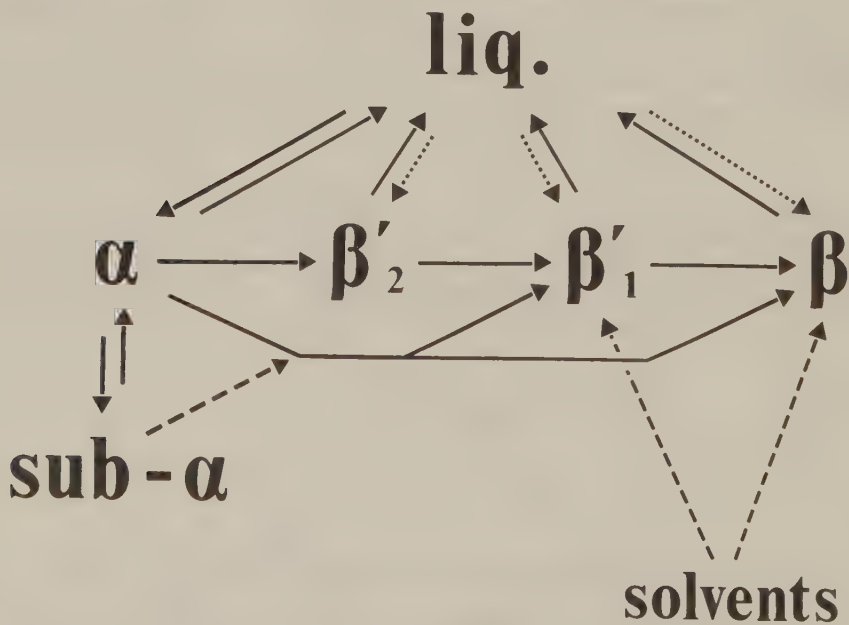


Fig. 27 Complete picture of the polymorphic transitions of triglycerides. All transitions are irreversible except the $\alpha \rightarrow \text{sub-}\alpha$ transition. The dotted lines indicate the possibility of a direct crystallization in other forms than the α -form. The possibility of growing crystals from solvents is also indicated.

The stability of the polymorphic forms, present in a fat, is also due to the purity of the system. The kinetics of the polymorphic transition are very important from a technical point of view (e.g. the $\beta' \rightarrow \beta$ transition influences the crystal size in margarine). Some work done so far concerns the $\beta' \rightarrow \beta$ transition of simple triglycerides (45,46). Recently the rate of the β -phase formation for 1-O-hexadecylglycerol was investigated in detail (47), using a position sensitive detector connected to a X-ray diffraction camera. It is obvious that fast detection is desirable, when the kinetics of polymorphic transitions are studied. Metastable forms can otherwise exist without being detected, due to experimental limitations.

Sometimes triglyceride systems are considered to show "memory" in the melt, due to crystal forms earlier present in the system. A true structural memory is not probable in the liquid state. A triglyceride system containing a few percent of tristearin, however, could contain β or β' microcrystals in the liquid, which are of micellar size (provided that the temperature is below the β -melting point). This explanation is therefore more probable.

The polymorphic behaviour of complex triglycerides

When a fat crystallizes, a small fraction can determine the overall crystallization. Even if technical fats consist of many different triglycerides, their crystallization behaviour can still be understood in terms of the triglyceride fraction that dominates the polymorphic behaviour. Studies of the polymorphic behaviour of pure triglyceride systems, using simple triglycerides, can therefore give useful results. Furthermore the possibility to study the polymorphic behaviour of complex triglycerides formed by interesterification of simple triglycerides seems to give additional useful information (III).

Technical applications

The functional properties of fats, *i.e.* the degree of dispersion, the solid-liquid ratio and the polymorphic behaviour, are important in food systems. Only margarine and chocolate will be discussed here, although the conclusions are valid also in other food systems.

Margarine

An ordinary margarine emulsion consists of water droplets and fat crystals in a continuous oil phase. The w/o-emulsion is stabilized by polar lipids (usually lecithin), and the fat crystals present in the system. The water phase is usually skim-milk. A phase diagram of relevance for the margarine emulsion is a ternary system polar lipids/triglycerides/water. Such a diagram was examined and found to show the existence of an L2-phase all up to the triglyceride corner (IV). It is therefore probable that an L2-phase is formed in the margarine process. In this connection

it should be pointed out that there is a close relation between triglycerides in the liquid state (discussed above) and the L2-phase (IV). The presence of fat crystals is necessary in order to obtain a stable margarine emulsion. By selecting the oils and fats (usually hydrogenated oils), that constitute the oil phase, carefully, physico-chemical properties (*i.e.* solid-liquid ratio and polymorphic behaviour) as well as nutritional aspects can be optimized. The solid-liquid ratio is directly coupled to the texture of the margarine (the more solid phase the harder the margarine), but the polymorphic behaviour is also of critical importance.

When a margarine is produced, the fat first crystallizes in the α -form, which almost directly is transformed into the β' -form. If, however, there is a transition into the β -form, a product which is unacceptable due to its texture is obtained. The reason is the size of the crystals, resulting in a sandy feeling in the mouth (48).

Hydrogenated rapeseed oil with low content of erucic acid (LOBRA) shows a strong tendency for a rapid transition into the β -form (V) (49). Due to nutritional reasons, the content of erucic acid in rapeseed oil grown in Sweden has been reduced by plant breeding from about 50% down to 1%. This change has given a very homogeneous fatty acid patterns, with C_{18} acids constituting nearly 90% of the acyl groups (V). Due to the homogeneous fatty acid pattern, the β -form is developed very fast in hydrogenated LOBRA, whereas the earlier type of rapeseed oil gave fats enough stable in the β' -form (49). One aim with this work has been to solve this problem.

The polymorphic behaviour of a LOBRA oil hydrogenated to a melting point of 40°C (LOBRA 40) was therefore first studied (V). By cooling the sample rapidly (about 10°C/min) down to 23°C, the α -form was found to be transformed within 5 min to the β' -form. The $\beta' \rightarrow \beta$ transition then started within 3 hours, and after about 10 hours practically all crystals were transformed to the β -form.

Two general types of β' -structure stabilization may be expected to delay the $\beta' \rightarrow \beta$ transition: variation of chain lengths, giving a disordered chain region near the methyl end group planes, or stabilization of the orthorhombic chain packing by solubilization into the lattice of triglyceride molecules which favours this chain packing.

Variations in triglyceride chain lengths must result in a crystal packing where a zone near the methyl end group planes is highly disordered, provided that mixed crystals are formed. There is crystal structure evidence for the existence of disordered hydrocarbon chains near the methyl end groups. The potassium caprate has been reported to exhibit an A $\rightarrow$ C transition at 76°C, where the unit cell dimensions are almost unchanged beside an elongation in the long-spacing direction, suggesting a partial "melting" of the chains near the methyl end groups (50). If there is a high degree of disorder in the methyl end group region, it should be expected that the energy difference between the β' - and β -crystal forms will be reduced, and therefore also the driving force towards a $\beta' \rightarrow \beta$ transition.

Two interesterified systems of triolein-tristearin and triolein-trilaidin have been mixed and studied with DPT (III). This mixture is quite similar to the LOBRA 40, both are plastic fats containing a large extent of *trans* fatty acids. Consequently, the polymorphic behaviour is quite similar in the two systems. If the interesterified system is heated slowly ($0.4^{\circ}\text{C}/\text{min}$) after rapid cooling ($80^{\circ}\text{C}/\text{min}$), the system undergoes the $\alpha \rightarrow \beta \rightarrow \text{liq.}$ transition. It is worth to notify that the interesterified system develops the β -form directly from the α -form without passing the β' -form. By blending the interesterified system with 2-stearo-dipalmitin (stable in the β' -form) the polymorphic behaviour of the entire system is changed. Now the $\alpha \rightarrow \beta' \rightarrow \text{liq.}$ transition takes place on slow heating. By introducing 2-stearo-dipalmitin into the system, an increased disorder in the methyl end group regions is probably obtained, which gives a stable β' -form. Thus blending LOBRA 40 with specific triglycerides might provide a solution to reduce the $\beta' \rightarrow \beta$ transition speed.

The second possibility is to use a lipid additive co-crystallizing with the fat, and favouring crystals with the orthorhombic chain packing ($0\perp$) rather than the triclinic chain packing ($T//$). Diglycerides are of particular interest in this respect, as they are known to be organized in an extended form (51). It should be emphasized that from a nutritional point of view diglycerides behave like a fat, being digested to the same components in the intestine. The advantage of diglycerides compared with additives such as sorbitan tristearate (earlier used for β' -stabilization (52)) is obvious. Strong stabilization effects of diglycerides on the β' -triglyceride crystal form were also observed (V). 1,2-Diglycerides prefer the orthorhombic chain packing and 1,3-diglycerides the triclinic. Consequently, 1,2-diglycerides stabilize the orthorhombic β' -form of triglycerides to a greater extent than 1,3-diglycerides. The pure LOBRA 40 begins to develop the β -form within 3 hours at 23°C . If the LOBRA 40 is blended with 5% of rac-1,2-dipalmitin, and treated in the same way as the pure LOBRA 40, the β' -form is stable for more than three days at 23°C . The amount of rac-1,2-dipalmitin can be kept quite low, in fact only 1% is needed to delay the $\beta' \rightarrow \beta$ transition enough. In this connection it should be mentioned that the effect of 1,3-diglycerides on the β' -stability of palm oil has been reported previously (53).

The effect of different chain lengths of the added diglycerides on the stabilizing effect is very pronounced. Since LOBRA 40 contains practically nothing but C_{18} chains (V), it is not surprising that distearin delays the $\beta' \rightarrow \beta$ transition even more than dipalmitin. Experiments with dieicosanoin showed a shorter delay in the $\beta' \rightarrow \beta$ transition than the addition of dipalmitin. Thus it seems that the maximal delay of the $\beta' \rightarrow \beta$ transition is obtained if the chain lengths of the added diglycerides are the same, or very similar, as the chain lengths of the actual triglycerides. The shorter chain length of the diglycerides, the smaller is the possibility to stabilize the orthorhombic form. If the diglyceride chains are too long in relation to the triglyceride chains, problems with co-crystallization probably will occur.

Not only the $\beta' \rightarrow \beta$ transition is influenced by diglycerides. Pure LOBRA 40 develops the β -form within 5 minutes at 23°C . The LOBRA 40 containing 5% of rac-1,2-dipalmitin shows an α -form that is stable for 20 minutes at 23°C as seen

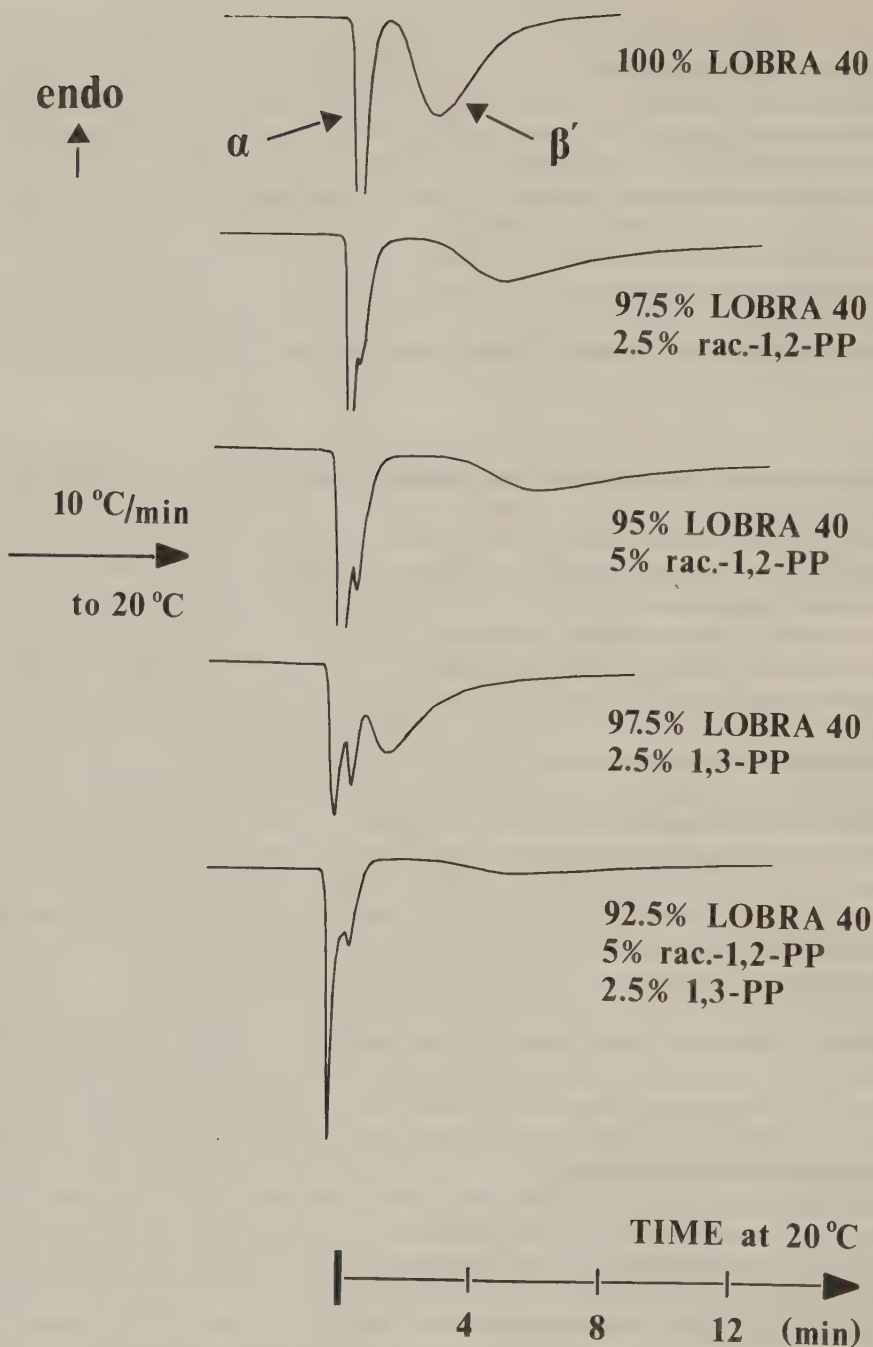


Fig. 28 DSC-thermogram of LOBRA 40 containing different proportions of rac-1,2-dipalmitin and 1,3-dipalmitin. All samples have been cooled (10°C/min) to 20°C and then the polymorphic transitions were followed isothermically.

with DPT (V). In order to study the influence of diglycerides on the $\alpha \rightarrow \beta'$ transition, various mixtures were analysed with DSC. The thermogram for LOBRA 40 containing different proportions of rac-1,2-dipalmitin and 1,3-dipalmitin is shown in Fig. 28. The samples have been cooled ($10^\circ\text{C}/\text{min}$) to 20°C , and then the thermal transitions are followed isothermally. The delay of the $\alpha \rightarrow \beta'$ transition, when rac-1,2-dipalmitin is introduced, is quite obvious. It can also be seen that 1,3-dipalmitin does not shift the $\alpha \rightarrow \beta'$ transition. When the peak of the α -crystallization is splitted, the reason is probably segregated crystallization caused by the diglycerides.

A pilot plant experiment based on these findings has also been performed (VI). A margarine blend consisting of LOBRA 34 and LOBRA 40 and soya oil with a solid-liquid ratio similar to an ordinary table-margarine was studied. The technical diglyceride blend used consisted of 68% diglycerides, 26% triglycerides and 6% monoglycerides. About 60% of the fatty acids were stearin and 25-30% palmitin, whereas about 60% of the diglycerides were in the 1,3-form and the rest 40% in the rac-1,2-form. The results are consistent with the laboratory experiments (V). Hence the potential of diglycerides as a β' -stabilizer of triglyceride systems is obvious.

Chocolate

Chocolate systems contain fat mainly in the solid state, and since the crystal forms should be metastable, the polymorphic behaviour of such fats is important. The fat can either be pure cacao butter or a mixture where cacao butter is partly or totally replaced by another fat.

A phenomenon sometimes occurring in the chocolate system is "bloom", *i.e.* a greyish surface, which is caused by polymorphic transitions (*cf.* 54). However, the classification of the various forms in relation to the structures discussed above is rather confusing (54-57).

The short-spacing data of the six forms of cacao butter, as reported in refs. (54,55), are compared with the short-spacing data of 2-oleo-distearin (sub- α , α and β) and 2-stearo-dipalmitin (β'_2 and β'_1) in Table I. The triglyceride composition in cacao butter is dominated by 2-oleo-distearin, 2-oleo-dipalmitin and 1-palmito-2-oleo-3-stearin, *i.e.* monounsaturated triglycerides with an oleic acyl group in the 2-position. Therefore 2-oleo-distearin is convenient to use as a reference, and 2-stearo-dipalmitin is used in order to get data on β'_2 and β'_1 .

On the basis of this comparison it is suggested that the six forms I-VI are identical to the forms discussed in this thesis, namely sub- α , α , β'_2 , β'_1 and β respectively. Since forms V and VI show the same short-spacing data, they might be the same forms. In a DSC work of Merker and Vaeck (57), cacao butter is suggested to show only four polymorphic forms. The polymorphic forms excluded should be number III (β'_2) and VI (the second β -form). These findings should be compared with problems involved in detecting different polymorphic forms with DSC (*cf.* Fig. 14) but also with the DPT studies of the interesterified system of triolein-tristearin (III).

Table 1. Classification of the polymorphic forms of cacao butter

I	sub- α	II	α	III	β_2	IV	β_1	V	VI	β
3.87(m)	3.78(w)					3.75(m)	3.86(s)	3.65(ms)	3.67(s)	3.64(m)
				3.87(w)	3.92(w)	3.88(w)	4.06(m)	3.73(ms)	3.84(m)	3.84(w)
4.17(s)	4.23(s)	4.20(vs)	4.25(s)	4.20(vs)	4.23(diff.s)	4.13(s)	4.22(s)	3.87(m)	4.01(w)	3.95(s)
						4.32(s)	4.36(m)	3.98(ms)	4.21(vw)	4.05(w)
								4.22(vw)		4.29(w)
								4.58(vs)	4.53(vs)	4.52(m)
								5.13(vw)	5.09(vw)	4.83(s)
								5.38(m)	5.37(m)	5.28(m)

It is possible that the result on diglycerides to hinder the $\beta' \rightarrow \beta$ transition could be used also in chocolate systems in order to avoid blooming. The diglycerides to be used in a cacao butter, however, should probably be different to the ones used in the case of margarine.

FUTURE APPLICATIONS

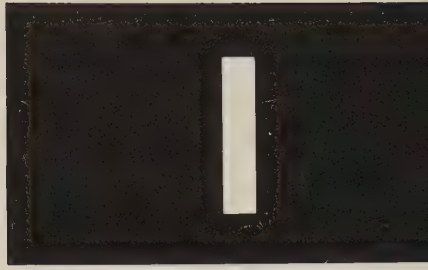
The polymorphic behaviour of fats can certainly be better understood if data on the kinetics involved in the polymorphic transitions and knowledge about the polymorphic behaviour of complex triglyceride mixtures are available.

Knowledge of the polymorphic behaviour could be used both in the plant breeding and in the technical processing. The possibility of avoiding *trans*-unsaturated fatty acids is one example. By total hydrogenation of a fat followed by interesterification with an actual oil a fat with suitable crystallization properties could be produced. For nutritional reasons (*cf.* Ref. (59)) the presence of *trans*-fatty acids in a margarine can thus be avoided.

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ON THE STRUCTURE OF TRIGLYCERIDES IN THE LIQUID STATE AND FAT CRYSTALLIZATION

by

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SUMMARY

Based on X-ray diffraction and Raman spectroscopy studies of trimyristin, a structure of triglycerides in the liquid state is proposed. The order in the melt is quite constant, even if the temperature is raised as much as 40°C above the melting point. The homologue series of simple triglycerides, together with 1-monomyristin and 1,2-dimyristin have been studied with the same techniques. The order of different segment in the hydrocarbon chains varies when different chain length, *i.e.* a long chain is more disordered at the methyl endgroup plane compared with a short one.

The crystallization of triglycerides is also discussed based on diffraction pattern *versus* temperature studies. Due to different cooling rates and different chain length a melt can crystallize not only in α but directly in β' or β .

INTRODUCTION

The crystal structure of the β -form of simple triglycerides has been known for a long time (1,2), and structures of the β' -form and the α -form were recently proposed (3). There is little information about the structure of triglyceride in the liquid state, however. Since the melt plays an important role when a fat crystallizes it is important to get a better understanding of the structure in the liquid state. The present work concerns the structure of the melt as studied by X-ray technique and Raman spectroscopy.

EXPERIMENTALS

Samples of trilaurin and tristearin were obtained from Fluka, tricicosanoin and tribehenin from Sigma. The samples of trimyristin, tripalmitin, 1-monomyristin and 1,2-dimyristin were prepared by standard methods. A diffraction pattern *versus* temperature (DPT) camera (4) was used. The X-ray films were examined with a photodensitometer. Raman spectra were recorded by a Dilor RTI 30 Laser Raman with triple monochromator using an Argon laser for excitation. Immediately prior to the diffraction and Raman studies all samples were heated in the same way *i.e.* heating for three minutes to a temperature of 30°C above the β -melting point, in order to destroy any "memory" of earlier crystals, and after that cooling to the temperature desired. When 1-monomyristin and 1,2-dimyristin were studied together with the homologue series of simple triglycerides the chosen temperatures were three degrees over the β -melting point of respective glyceride.

RESULTS AND DISCUSSION

In Fig. 1 the long-spacing data from the X-ray scattering studies of trimyristin in the liquid state are shown. The dominating intensity has been interpreted as the bilayer thickness (5), and this value is constant from 45°C to 80°C. This indicates that the main features of the structure of the triglyceride melt is quite constant within this temperature range.

In order to compare the melt of different glycerides the homologue series of simple triglycerides from tridodecanoin to tribehenin, 1,2-dimyristin and 1-monomyristin were studied. The result is shown in Fig. 2. The average thickness of the bilayer of the homologue series gives a curved line, *i.e.* the larger the hydrocarbon chain the smaller the contribution of the chain end to the d-value.

If the curve in Fig. 2 is extrapolated to chain length zero, the intercept should correspond to the space occupied by the glycerol groups and the gap over the methyl end groups between two adjacent chain layers.

Both 1-monomyristin and 1,2-dimyristin show higher d-value, 28.5 Å and 24.5 Å, respectively, compared with trimyristin, 22 Å. It seems natural to assume that mono- and diglycerides should give curves with the same shape as the one of triglycerides. The extrapolation to chain length zero should be expected to give a somewhat higher intercept for mono- and diglycerides due to the state of the glycerol residue, and this is consistent with the difference in d-value between 1-monomyristin, 1,2-dimyristin and trimyristin.

The half peak values (the line width at the half value of the intensity in relation to the background) for the different glycerides are given in Table I. The lower half peak values for the melt of 1-mono- and 1,2-dimyristin compared with the melt of

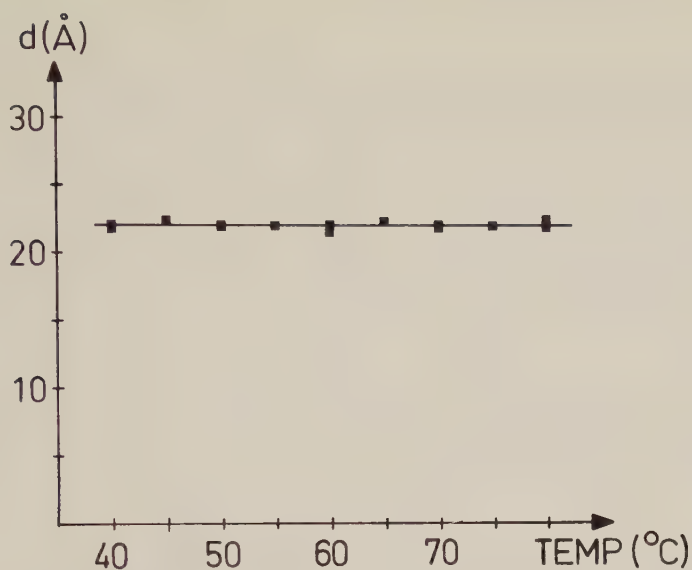


Fig. 1 The bilayer thickness in the trimyristin melt as a function of temperature.

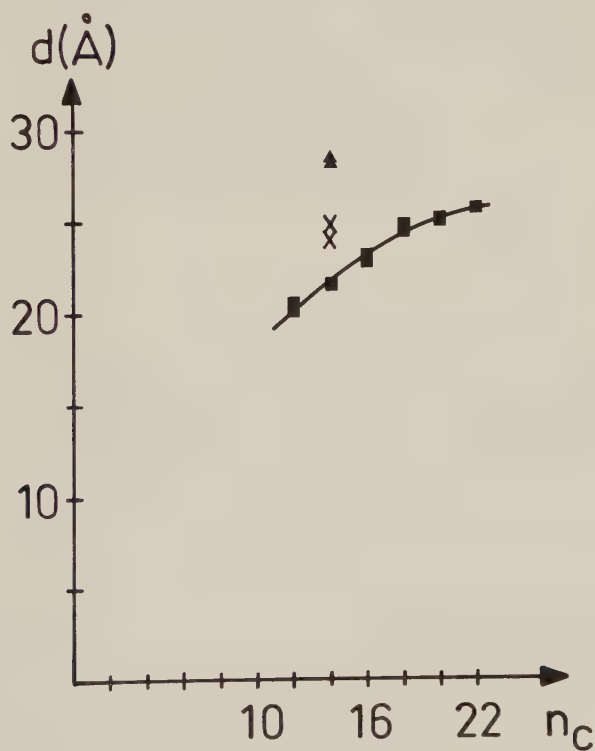


Fig. 2 The bilayer thickness in the melt of simple triglycerides (squares), 1-monomyristin (triangles) and 1,2-dimyristin (crosses). All samples were measured 3°C above respective β -melting point.

Table 1

Half peak values for 1-monomyristin, 1,2-dimyristin and trimyristin

Sample	Temp. (°C)	d (Å)	Half peak value (Å)
1-Monomyristin (liq)	73	28.5	19.5
1,2-Dimyristin (liq)	57	24.5	22
Trimyristin (the β -form)	25	36.2	13.5
Trimyristin (liq)	50	22.0	25
Trimyristin (liq)	80	22.3	26

trimyristin indicate that these structures are more rigid. This may be due to the existence of hydrogen bonds, which do not exist in the case of triglycerides.

Raman spectroscopy is an useful method in order to identify the different states of order in lipids (6,7). The C-C stretching vibration region is particularly powerful in order to identify whether the hydrocarbon chains are crystalline or liquid. Lipids with crystalline chains show two sharp peaks near 1065 cm^{-1} and 1130 cm^{-1} , whereas these peaks are replaced towards a broad band near 1090 cm^{-1} when the chains melt. The ratio $1080/1130$ has been taken as a measure of the *trans/gauche* ratio, *i.e.* the degree of liquid-type disorder.

The ratio $1080/1130$ for trimyristin in the liquid state is plotted in Fig. 3. The ratio $1080/1130$ is constant over the temperature range studied ($45\text{--}80^\circ\text{C}$), *i.e.* the chain

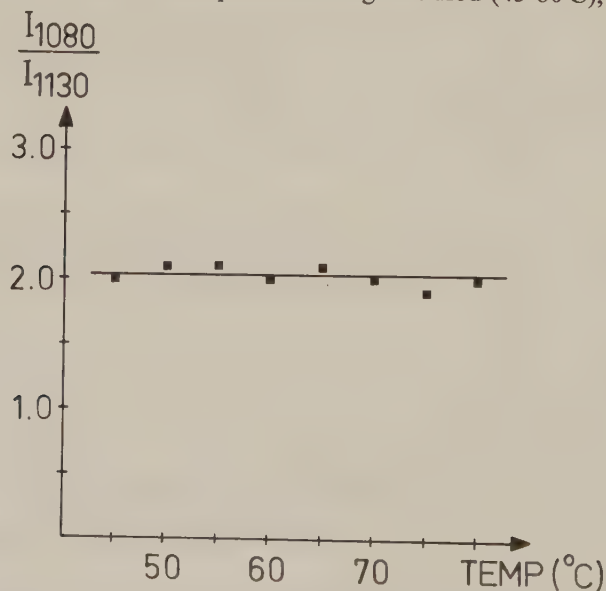


Fig. 3 Ratio of the 1080/1130 lines on the Raman spectra of trimyristin melt as a function of temperature.

disorder/chain mobility is constant. The result shown in Fig. 3 is in good correlation with the results shown in Fig. 1. Hence the order in the melt is more or less constant even if the temperature is elevated. It seems probable that the main change is the size of the regions arranged in lamellar units. In Table I the differences in half peak values for trimyristin at 50 and 80°C are shown. This change must be due to a reduction of the size of the lamellar units. Line broadening was recently used (8) to estimate the size of the domains in a L2 phase in a liquid crystalline phase. In this connection it should be pointed out that there is a close resemblance between a glyceride melt and a liquid crystalline phase.

The discussion above is visualized in Fig. 4. A dynamic model is proposed where size and orientation of the lamellar units vary with diffusion rates of the molecules.

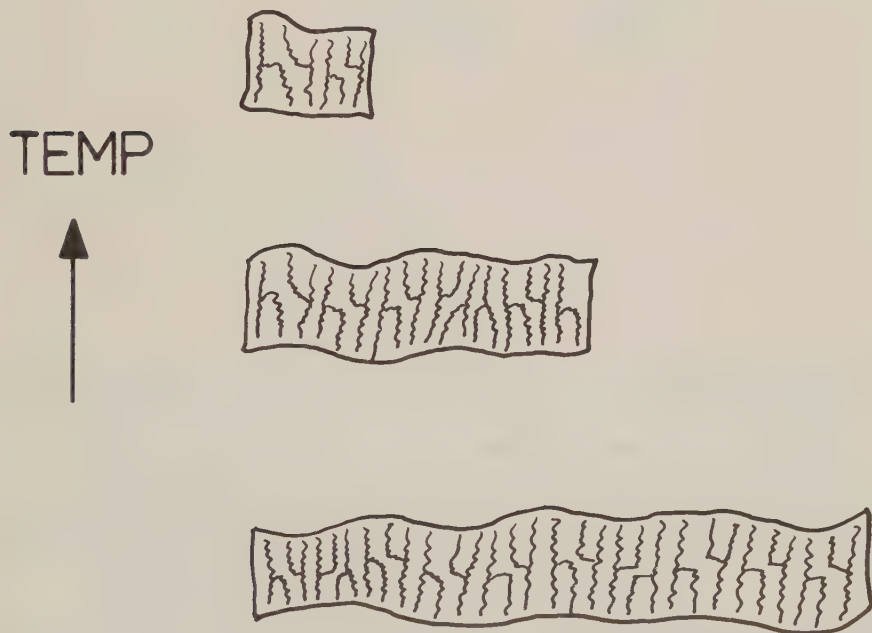


Fig. 4 Visualization at the reduced size of the lamellar units with temperature.

The ratio 1080/1130 of the Raman spectra for the homologue series of simple triglycerides from tridodecanoin to tribehenin, 1,2-dimyristin and 1-monomyristin, all liquid, are shown in Fig. 5. The ratio 1080/1130 is drastically changed when the chain length is increased, *i.e.* the *gauche* proportion is much higher for the long carbon chain compared with a short one. The curvature of the line in Fig. 2 is consistent with the drastic increase of the 1080/1130 ratio in Fig. 5, *i.e.* the structure of the methyl end group plane is much more disordered. The chains are fixed at the glycerol group, and the mobility is increased with the distance to the glycerol group.

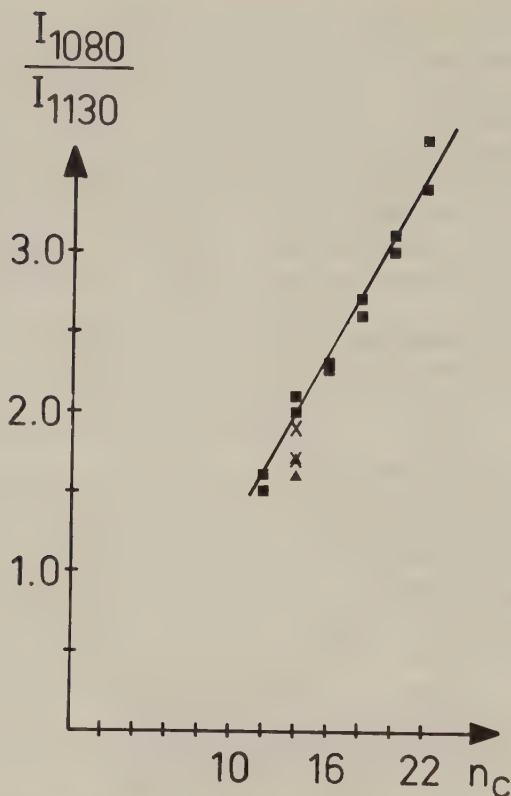


Fig. 5 Ratio of the 1080/1130 lines in the Raman spectra of the melt of simple triglycerides (squares), 1-monomyristin (triangles) and 1,2-dimyristin (crosses). All samples were measured 3°C above respective β -melting point.

It has been shown (9) for liquid crystals of lipids that due to the anchoring of one chain end at the lipid water interface, the *gauche* proportion (*i.e.* the chain mobility) is higher in the opposite chain end.

If trimyristin, 1,2-dimyristin and 1-monomyristin are compared, the *gauche* proportion is highest for trimyristin and lowest for 1-monomyristin. This result correlates well with the difference in bilayer thickness (Fig. 2) and band width (Table I) found in the X-ray studies.

When the temperature is decreased the lamellar units are increased in size until finally crystallization takes place. The probability of regions with neighbouring hydrocarbon chains orientated in all-*trans* also increases. When formation of adjacent all-*trans* chains within a lamellar unit dominates over formation of *gauche* conformation a crystal nucleus is obtained (see Fig. 6).

Crystallization can occur if the temperature is lower than the β -melting point. If a melt of trimyristin is cooled rapidly (80°C/min), the α -form is formed (see Fig. 7). When the temperature is increased the α -form is transformed to the β -form. If

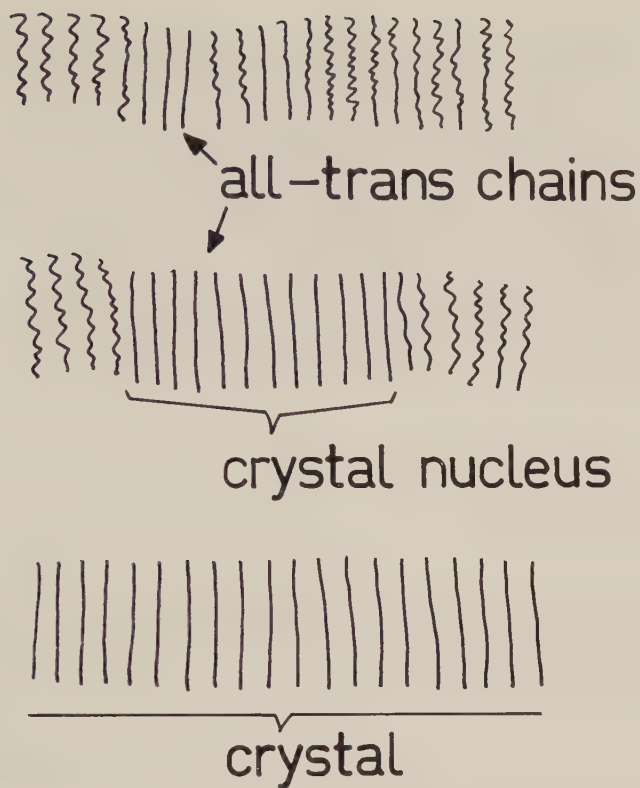


Fig. 6 Proposed mechanism for the crystallization of a triglyceride melt.

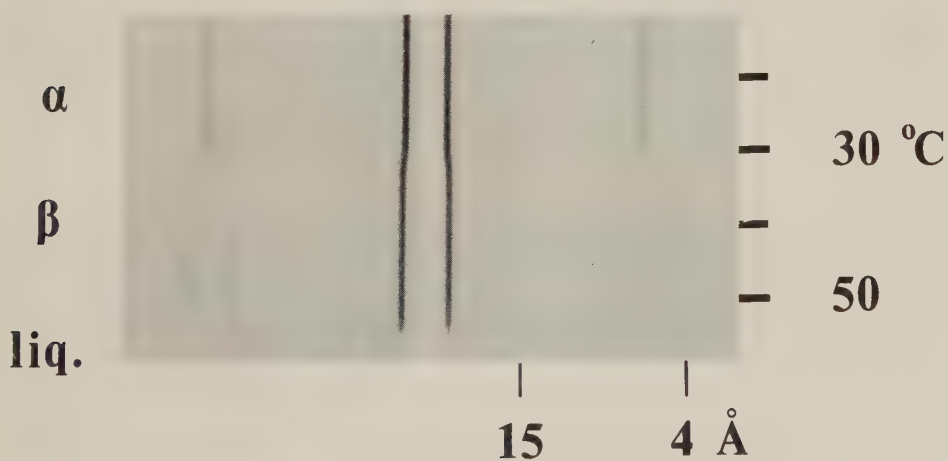


Fig. 7 DPT-diagram of the polymorphic transitions of trimyristin when the α -form is heated ($0.4^\circ\text{C}/\text{min}$).

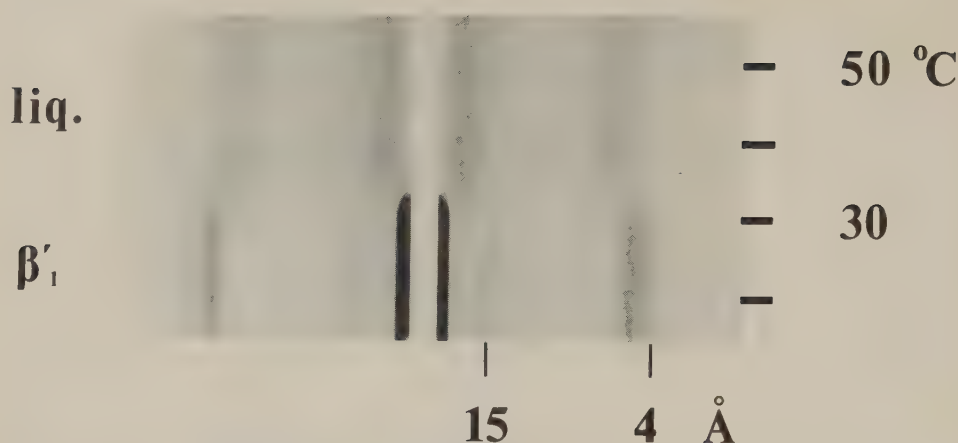


Fig. 8 DPT-diagram of the polymorphic transitions of trimyristin when the melt is cooled ($0.4^{\circ}\text{C}/\text{min}$).

the same melt of trimyristin is cooled slowly ($0.4^{\circ}\text{C}/\text{min}$) it crystallizes directly in the β' -form (see Fig. 8). The reason why trimyristin develops the β' -form directly without passing the α -form is due to the fact that crystallization nuclei are formed already above the α -melting point.

In Fig. 9 the crystallization pattern at slow cooling is shown for some simple triglycerides. In the case of trimyristin, the α -form might exist and then be transformed directly to the β' . For trilaurin, however, it is obvious that the α -form does not exist before the crystallization in the β' -form. Since trilaurin crystallizes 15°C above its α -melting point the chain mobility is so high that the $\beta' \rightarrow \beta$ transition occurs at 21°C instead of the β' -form being stable. The reason why tristearin crystallizes in the α -form under these conditions can be due to the lower probability that a long chain should develop all-*trans*. By holding isothermally a tristearin melt in the undercooled region above the α -melting point, *e.g.* at 60°C in order to increase the probability for crystallization, the β -form is developed directly within 38 minutes.

A technical fat, to be used in the margarine production, is plastic. It consists of a mixture of various triglycerides. Some of these triglycerides have a melting point that is very high. If the time in the undercooled region is long enough these high melting triglycerides could segregate and crystallize directly in the β -form as small crystallites, which can influence the crystallization of the entire fat.

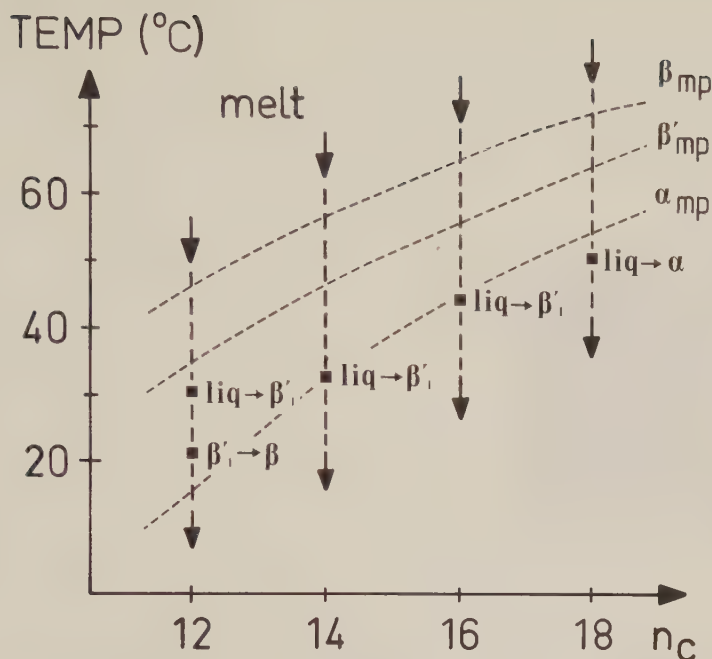


Fig. 9 Polymorphic transition for the melt of simple triglycerides when slowly cooled ($0.4^{\circ}\text{C}/\text{min}$, studied with DPT).

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On the Crystal Structure of the β' -Form of Triglycerides and Structural Changes at the Phase Transitions $LIQ. \rightarrow \alpha \rightarrow \beta' \rightarrow \beta$

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Based on X-ray single crystal data of the β' -form of triundecanoin the general features of the molecular arrangement in this crystal form have been derived. The unit cell contains eight molecules arranged according to space group $P2_1/c$, with alternation of the tilt of the hydrocarbon chains in adjacent bimolecular layers. The structure within the molecular layer is quite similar to that of the β -form; the main difference being the chain packing. A second β' -form, β'_2 , of triundecanoin has been observed and it differs from the one mentioned above in the orientation of the chain packing subcell 0_1 in relation to the true unit cell. The polymorphic transitions have been followed by recording of the diffraction pattern versus temperatures. The polymorphic transitions $\alpha \rightarrow \beta' \rightarrow \beta$ can be regarded mainly as different lateral arrangement of a dimeric unit. In the α -form the chains are disordered near the methyl end groups, and due to this disorder the structure is closely related to the lamellar liquid-crystalline phase. It is also possible to classify β' -forms according to short-spacing data into a β'_1 -type and a β'_2 -type, and these groups can be identified also in complex triglycerides.

Introduction

The crystal structure of the β -form of simple triglycerides has been known for a long time^{1,2}, and an important generalization towards complex triglycerides was recently reported by S. de Jong³. There are little information in the

Über die Kristallstruktur der β' -Form von Triglyceriden und Strukturumwandlungen bei Phasenübergängen $LIQ. \rightarrow \alpha \rightarrow \beta' \rightarrow \beta$

Auf Grund von Röntgenuntersuchungen über Ein-Kristall-Daten der β' -Form des Triundecanoin wurden die allgemeinen Merkmale der Molekülanordnung erhalten. Die Elementarzelle enthält acht Moleküle, entsprechend zur Symmetrie $P2_1/c$, mit Ausrichtung auf die wippende Stellung der Hydrocarbon-Ketten in den nahegelegenen bimolekularen Schichten. Die Struktur in der Molekularschicht ist sehr ähnlich der β -Form — der Hauptunterschied liegt in der Kettenpackung. Eine zweite β' -Form, β'_2 des Triundecanoin ist beobachtet worden, die sich von der obengenannten in der Orientierung der Kettenpackung der Unterzelle 0_1 im Verhältnis zu der richtigen Elementarzelle unterscheidet. Die polymorphen Umwandlungen wurden durch Registrierung der Diffraktionsmuster gegen die Temperaturen verfolgt. Die polymorphen Umwandlungen $\alpha \rightarrow \beta' \rightarrow \beta$ können hauptsächlich als unterschiedliche Seitenanordnungen der dimetrischen Einheit angesehen werden. In der α -Form befinden sich die Ketten ungeordnet in der Nähe der Methyl-Endgruppen, und auf Grund dieser Unordnung ist die Struktur nahe an die Flüssigkeit-Kristall-Phase geknüpft. Es ist ebenfalls möglich, die β' -Formen entsprechend der Röntgenkleinwinkel-Daten in eine β'_1 -Type und eine β'_2 -Type zu klassifizieren, und diese Gruppen können auch in komplexen Triglyceriden identifiziert werden.

literature on the structure of the α - and β' -forms, however, although these forms are of biological as well as of technical importance.

Triundecanoin gives a stable β' -form from solvents, and single crystals of good quality can be grown. The present

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¹ L. H. Jensen and A. J. Mabis, Nature [London] **197**, 681 [1963].

² K. Larsson, Proc. Chem. Soc. **87** [1963].

³ S. de Jong, Triacylglycerol Crystal Structures and Fatty Acid Conformations, Thesis, University of Utrecht, 1980.

work concerns the structure of this β' -form. Furthermore the structural relationships between the different polymorphic forms have been analysed by X-ray diffraction and Raman spectroscopy.

Experimental

The sample of triundecanoin used has been described earlier⁴. Crystals for single-crystal work were grown from acetone at 20° C. Samples of triundecanoin and tristearin were obtained from Fluka, tritridecanoin, tripentadecanoin and triheptadecanoin from Analytical Standards, Gothenburg, Sweden. The sample of 1,3-dipalmitoyl-2-stearoyl glycerol was an own preparation. A Weissenberg camera was used for the X-ray single-crystal work, and a diffraction — pattern — versus — temperature (DPT) camera⁵ was used to study the polymorphic transitions. Raman spectra were recorded by a Cary 82 spectrophotometer using an argon laser for excitation (cf. Ref. 6).

Results and Discussion

1. The stable β' -form of triundecanoin (β')

The unit cell is monoclinic with the following dimensions: $a = 23.6 \pm 0.2 \text{ \AA}$, $b = 5.69 \pm 0.05 \text{ \AA}$, $c = 58.5 \pm 0.5 \text{ \AA}$, $\beta = 90^\circ \pm 0.5^\circ$ C. It contains eight molecules, and according to the absent reflexions $h0l: l=2n+1$ and $0k0: k=2n+1$, the space group is $P2_1/c$. There are thus two molecules in the asymmetric unit. From X-ray powder diffraction data it was determined that this crystal form is a β' -form. Thus it exhibits two dominating short-spacing lines at 4.32 and 3.96 $\text{\AA}$, characteristic for the orthorhombic chain packing (01).

The chains are tilted in the (100)-projection as well as in the (011)-projection and close-packed according to the orthorhombic chain packing, 01. The chain packing subcell is oriented in relation to the unit cell so that the (001)-plane of the crystal is parallel to the (111)-plane of the orthorhombic subcell. The Weissenberg photographs of the (h0l)- and (h1l)-reflections, respectively, are shown in Fig. 1. There is a close structural relation between this (010)-projection of the structure and the (100)-projection of the structure of the β -form^{1,2}. The hydrocarbon chain structure of the (010)-projection is seen in Fig. 2. The detailed structure of the glycerol groups is not known, and only their location has therefore been indicated. The positions of the chain axes (see Fig. 3) and the space available, however, indicate that the glycerol group region can have the same structure as in the β -form. As the orthorhombic chain packing requires an even number of chains laterally arranged in the unit cell (due to two independent zig-zag planes) there are six chains in each hydrocarbon chain layer of the unit cell.

Thus, even if the glycerol group structure would be identical to that of the β -form, there must still be two molecules in the asymmetric unit due to this consequence of the 01-chain packing. Beside this difference from the β -form (with one zig-zag plane and therefore only three chains per chain layer) the structure of the (010)-projection of the β -form and of this (010)-projection are closely related.

The (100)-projection, however, shows a structure quite different from that of the β -form. The chains are tilted in relation to the end-group planes, and from the space-group

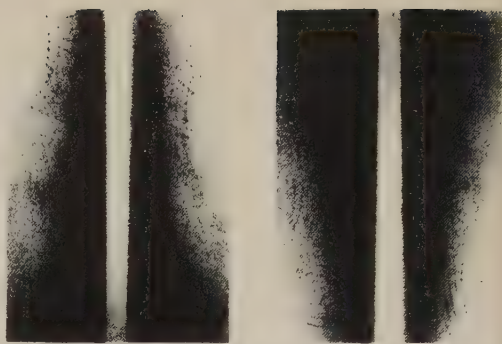


Fig. 1. Weissenberg photographs of the (h0l)-zone (to the left) and (h1l)-zone (to the right) of the β' -form of triundecanoin; the high-angle part, where no reflexions are visible, is not shown

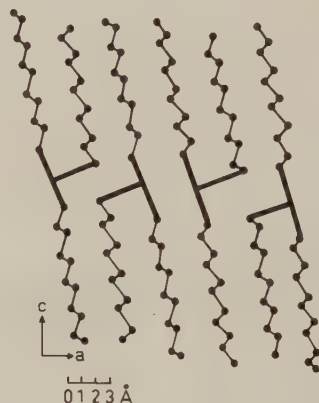


Fig. 2. Main features of the structure of the (010)-projection of the β' -form of triundecanoin; the glycerol group skeleton is indicated by thick bars

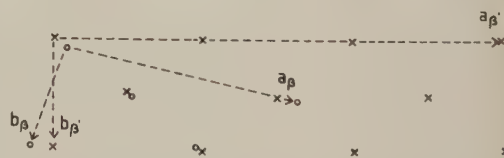


Fig. 3. Geometric relations between the (ab)-lattices in the β' - and β -forms, and the chain axes positions in the β -form (circles) and β' -form (crosses)

symmetry, it follows that there are two chain tilt directions. The double-chain layers location between $z = -1/4$ and $z = +1/4$ are thus tilted in an opposite direction, compared to the double-chain layer located between $z = 1/4$ and $z = 3/4$. The question if the chain tilt alternates in the glycerol cannot be unambiguously determined from the X-ray data. In an earlier discussion of the structure of the β' -form⁴, the space group was proposed to be $P2_12_12_1$. The present single-crystal data, however, show that this apparent orthorhombic symmetry was due to twinning. As the dimensions correspond to an orthorhombic unit cell such twinning is strongly favoured. An earlier interpretation of alternation of chain tilt at the glycerol groups⁴ was based on the space

⁴ K. Larsson, Arkiv Kemi **28**, 35 [1964].

⁵ E. Stenhagen, Acta Chem. Scand. **5**, 805 [1951].

⁶ K. Larsson, Chem. Phys. Lipids **10**, 165 [1973].

group P2₁2₁2₁. According to the present data, however, it is possible that molecular double-chain layer of the β' -form can have the same general structure as that of the β -form. With regard to the observed successive changes of the polymorphic transitions described below, it is natural to assume that the change of chain tilt takes place at the methyl end group plane (see Fig. 4). As evident from Fig. 3



Fig. 4. Chain directions in the (100)-projection of the β' -form of triundecanoin indicated by one molecule in each molecular layer of the unit cell

it is possible that the chain-axes only need minor positional changes at the $\beta' \rightarrow \beta$ transition. The angle of tilt is also very close in the two forms (the double-layer thickness for the β' - and β -forms of triundecanoin are 29.5 Å and 29.6 Å, respectively).

2. Structural relations between the polymorphic forms α , β' , β'_2 and β

A dynamic, mainly lamellar, type of structure was proposed for the liquid state of simple triglycerides just above the melting point⁷ based on X-ray scattering data (see Fig. 5). Such an order in the liquid state is also consistent with the experience that a fat should be brought up to a temperature far above its melting point (at least 30°C), in order to avoid that structural 'memories' from earlier crystals will influence the new crystallization. The arrangement in lamellar units should thus be expected to break down successively with increasing temperature of the melt towards dimers, and perhaps even further, with randomly distributed monomers. Observations on molecular motion in a triglyceride melt might be related to different degrees of lateral molecular arrangement (cf. Ref. ⁸).

The transitions from the α -form into the β' -form and finally into the β -form are irreversible, and α must always be passed to get β' , and in the same way the β' -structure must pre-exist in order to get the β -form. This should be expected if there are structural features that persist through

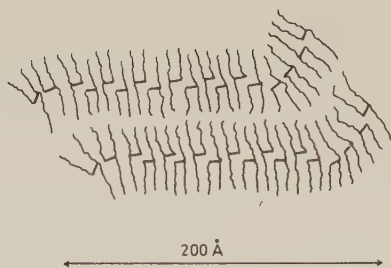


Fig. 5. Order proposed to exist in the liquid state of triglycerides just above the melting point⁷

the transitions, and the arrangement into similar molecular dimeric units is proposed to be the reason.

With the order in the melt shown in Fig. 5, the dimeric units exist already when the liquid approaches the crystallization temperature. The formation of the α -form can be regarded as a first step in finding the best chain arrangement. The shortspacing data show that the chains are arranged according to the hexagonal packing (H), with a high degree of oscillational mobility of the chains (cf. Ref. ⁸). The dimension changes with temperature were studied, and a DPT-diagram and densitograms of tristearin are shown in Figs. 6 and 7. There is a minor shift in the 4.2 Å spacing, 4.12 Å at 0°C and 4.18 Å at 50°C. The bilayer thickness $d(001)$ was found to be 51.6 Å at 0°C and 50.6 Å at 50°C. A reduction of this size of order of the long-spacing with temperature was the main reason for suggesting a liquid state of the hydrocarbon chains of liquid-crystals in the classical work by V. Luzzati *et al.*⁹

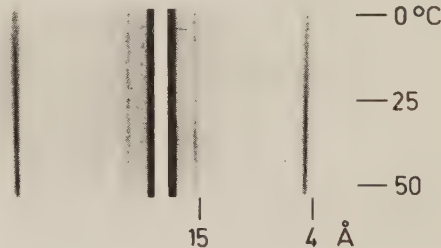


Fig. 6. DPT-diagram of tristearin in the α -form during heating 0.4°C/minute

Raman spectroscopy is a useful method in order to identify the different states of order of lipids^{6,10}. The C—C stretching vibration region is particularly powerful in order to identify whether the chains are crystalline or liquid. We have recorded Raman spectra during the phase transitions, and the C—C stretching vibration region was analysed. Lipids with crystalline chains show two sharp peaks near 1065 and 1130 cm^{-1} , whereas these peaks are replaced towards a broad band near 1090 cm^{-1} when the chains melt. The ratio 1080/1130 cm^{-1} is thus a measure of the degree of liquid-type of disorder. As can be seen in Fig. 8 there is a significantly 'liquid' contribution in the spectra of the α -form compared to the β' - and β -forms. The basis of

⁹ V. Luzzati, H. Mustacchi, A. Skoulios and F. Husson, *Acta Cryst.* **13**, 660 [1960].

¹⁰ I. L. Lippert and W. L. Peticolas, *Proc. Nat. Acad. Sci. USA*, **68**, 1572 [1971].

⁷ K. Larsson, *Fette · Seifen · Anstrichmittel* **74**, 136 [1972].

⁸ J. Clifford, *Nature [London]* **195**, 568 [1962].

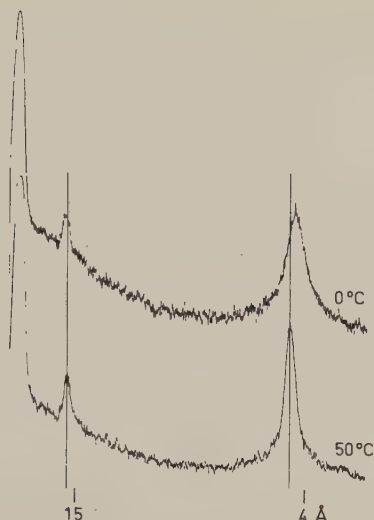


Fig. 7. Photodensitometer tracings (densitograms) of the α -form of tristearin from the DPT-diagram of Fig. 6

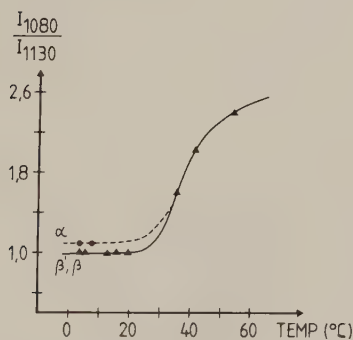


Fig. 8. Ratio of the 1080/1130 cm^{-1} lines in the Raman spectra of the different polymorphic forms of low-erucic rapeseed oil hydrogenated to a melting point of 40° C¹³

the spectral interpretation is based on *trans/gauche* conformation of the chains¹⁰, and although there is an oscillational chain disorder in the α -form it can hardly be space for *gauche*-conformation in any chain segment.

M. van den Tempel¹¹ has reported that the α -form differs from ordinary crystals by not showing super-cooling, and in this respect it behaves like the smectic (lamellar) form of liquid crystals. On the basis of the X-ray diffraction and Raman spectroscopy data given above, the main features of the molecular packing are proposed to follow the structure model given in Fig. 9. Thus the molecular dimeric units are of the same type as in the β - and β' -forms (Fig. 2), with similar head-to-head packing of the glycerol groups and the same chain axes directions. The end-group region, however, has disordered chains just like the lamellar liquid-crystalline phase. The physical properties of the α -form should therefore be expected to be related to those of

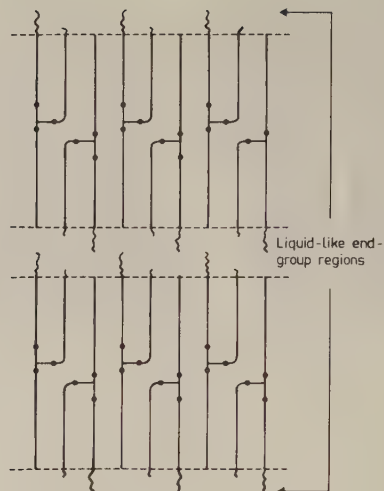


Fig. 9. Proposed structure of the α -form of triglycerides projected along the short orthorhombic axis a_0 (5 Å)

The chain axes are indicated; the main part of the hydrocarbon chains are oscillating and hexagonally close-packed; the methyl end group regions are somewhat more disordered, as in liquid crystals

lamellar liquid crystals. The resistance against deformation parallel to the bilayer planes should thus be expected to be very low.

This geometry of the lattice of the α -form is also consistent with the observed X-ray spacings. The long-spacing data of the different polymorphic forms are plotted in Fig. 10, and it can be seen that the intercept obtained by extrapolation to chain length zero is much larger in the α -form compared to the β'_1 - and β'_2 -forms.

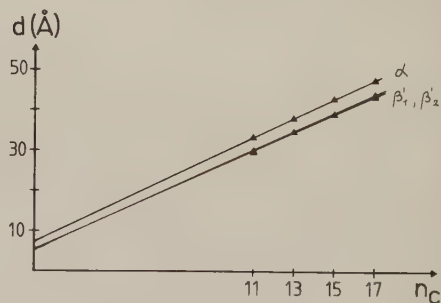


Fig. 10. Long-spacing of the different forms of odd triglycerides plotted against acyl chain length

This intercept corresponds to the space occupied by the glycerol groups and the gap between the methyl end groups in adjacent layers. Only a minor proportion of this difference can be due to the difference in chain tilt (90° C for the α -form and near 70° C for the β'_1 - and β'_2 -forms). The structure proposed in Fig. 9, however, requires a less dense packing in the methyl end group region, due to the liquid disorder. The higher value of the α -form intercept is therefore in agreement with the proposed structure. A transition which always seems to take place, when a form with hexagonal chain packing is cooled, is that the chain

¹¹ M. van den Tempel, Physicochimie des Composés Amphiphiles, Colloques Nationaux du C. N. R. S. 938, 261 [1979].

packing goes over 0L. This transition was first followed in *n*-paraffins by A. Müller¹², and the dimensional changes involved are shown in Fig. 11. There are reasons to expect that the chain packing changes in the same way at the transition $\alpha \rightarrow \beta'$.

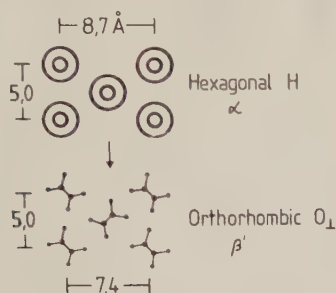


Fig. 11. Dimensional changes when the hexagonal packing of the α -form is transformed into the orthorhombic chain packing of the β' -form; the lattice contracts in one direction as evident from the change 8.7 Å to 7.4 Å

A DPT-diagram of triundecanoin is shown in Fig. 12, and photodensitometer tracings of the intensities of the different forms are given in Fig. 13. These data show the existence of two β' -forms. The polymorphism of tritridecanoin, tripentadecanoin and triheptadecanoin were also studied. All these homologues show the same behaviour, giving both a β'_2 -form and a β'_1 -form (see Table 1). In Fig. 15 the thermal transitions for triundecanoin, tritridecanoin, tripentadecanoin and triheptadecanoin are shown (the transition temperatures are based upon the DPT recordings of the same type). The samples were heated at a constant rate of 0.4° C/min. According to an earlier proposed nomenclature¹², the transitions evident from Fig. 12 are $\alpha \rightarrow \beta'_2 \rightarrow \beta'_1 \rightarrow$ liquid. The background to the different intensity distribution of the two β' -forms can be explained in the following way:

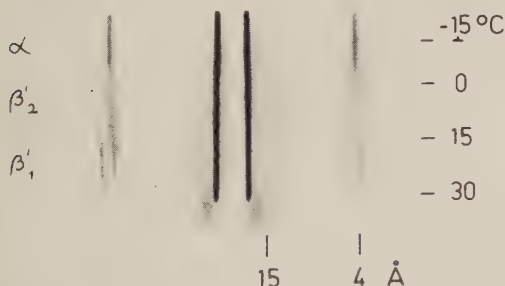


Fig. 12. DPT-diagram of the polymorphic transitions of triundecanoin when the α -form is heated (0.4° C/minute)

The dominating short-spacing lines from the β' -form correspond to the interplanar distances of the subcell $b/2$ ($= 3.8$ Å) and the a_b diagonal/ 2 ($= 4.2$ Å) (see Fig. 11). The orientation of the subcell within the true unit cell will also influence the intensities of the observed short-spacing lines, and an example of one possible orientation is shown in Fig. 14. This figure illustrates the orientation of the chain packing subcell so that the subcell plane (011) is parallel to

¹² A. Müller, Proc. Roy. Soc. London A 127, 417 [1930].

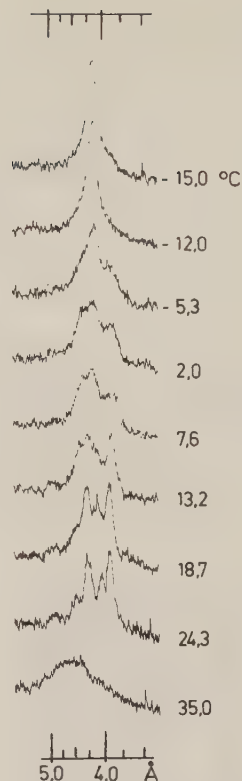


Fig. 13. Densitograms recorded at different temperatures of DPT-diagram of Fig. 12

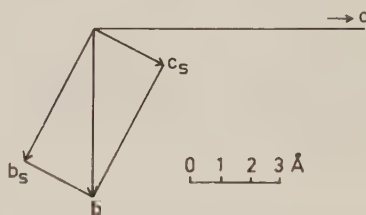


Fig. 14. A projection of unit cell along the a -axis and an orthorhombic chain packing subcell oriented with the c_b diagonal coinciding with the b -axis

plane (001) of the true unit cell (this mutual orientation is usually described as $0L[011]$). With such an orientation, the interplanar spacing $b/2 = \sqrt{b_s^2 + c_s^2}/2 = 4.0$ Å will give rise to a strong X-ray reflexion intensity. We should thus expect to observe a 4.0 Å short-spacing line, due to the subcell orientation, in addition to the 3.8 and 4.2 Å lines. The β'_1 -form described above has the orientation of the $0L$ subcell defined by $0L(111)$. This orientation should analogously be expected to give short-spacing lines at 4.0 Å ($= \sqrt{b_s^2 + c_s^2}/2$) and at 4.5 Å ($= \sqrt{a_b b_s^2 + c_s^2}/2$ with $a_b b_s$ = subcell diagonal). The orientation $0L(101)$ should be expected to give an additional line at 4.3 Å, which is in

¹³ L. Hernqvist, B. Herslöf, K. Larsson and O. Podlaha, J. Sci. Food Agric. 32, 1197 [1981].

Table 1

Short-spacings of the β'_2 and β'_1 forms of some odd members of simple triglycerides

Triundecanoin	β'_2	3.91 m	4.22 s	4.36 s	
	β'_1	3.96 s	4.08 m	4.32 s	4.52 w
Tridecanoin	β'_2	3.92 w	4.26(diff.)s		
	β'_1	3.87 s	4.09 m	4.29 s	4.45 w
Tripentadecanoin	β'_2	3.91 m	4.15 s	4.32 s	
	β'_1	3.93 s	4.07 m	4.26 s	4.41 m
Triheptadecanoin	β'_2	3.92 m	4.22 m	4.37 s	
	β'_1	3.93 s	4.15 m	4.33 s	4.51 w

s = strong; m = medium; w = weak

agreement with the observed short-spacing of β'_2 (see Table 1). It is proposed that for the β'_2 the chain packing end group plane is $0\perp(101)$, according to the short-spacing data and the angle of tilt according to the long-spacing. The angles of tilt of the chains differ only a few degrees between these two $0\perp$ chain arrangements in β'_1 and β'_2 (about 71°C and 74°C , respectively, which is consistent with the long-spacings 29.9 Å and 30.3 Å respectively).

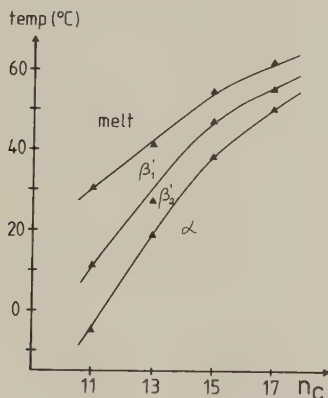


Fig. 15. Transition temperatures of odd mono-acid triglycerides

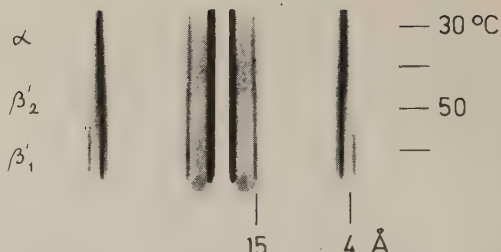


Fig. 16. DPT-diagram of the polymorphic transitions of 1,3-dipalmitoyl-2-stearoyl glycerol when the α -form is heated ($0.4^\circ\text{C}/\text{minute}$)

The following short-spacing lines were obtained: $\alpha = 4.18$ (diff.) s; $\beta'_2 = 3.92$ w, 4.23 (diff.) s; $\beta'_1 = 3.86$ s, 4.06 m, 4.22 s, 4.36 m

It should also be pointed out that the extra short-spacing lines discussed above, which are due to the fit of the subcell into the true unit cell, can be absent for symmetry reasons (space-group of the true unit cell), and therefore it is not enough to know the short-spacing lines for determination of the chain packing end-group plane. For this reason it is in some cases hard to identify β' -forms from the short-spacing lines, but an analysis of the extra lines and their origin (as discussed above), the β' -identification can be done unambiguously. It is also possible to use the end-group plane classification of β' -forms to obtain information of the geometry of methyl end-group terraces, and to find out which mixed triglycerides that fit best into the β'_1 - or the β'_2 -form. An illustration of a mixed triglyceride is given in Fig. 16. This type of analysis will be continued by one of us (L. Hernqvist) in a similar manner that S. de Jong used in his thesis³.

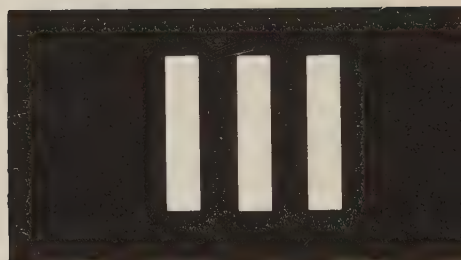
Note added in proof

In parallel with this work T. D. Simpson and J. W. Hagemann have reported two β' -forms for tristearin (J. Amer. Oil Chemists' Soc. **59**, 169 [1982]), which is consistent with our data.

Acknowledgment

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POLYMORPHISM OF INTERESTERIFIED TRIGLYCERIDES AND TRIGLYCERIDE MIXTURES

by
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SUMMARY

Complexes of well defined triglycerides have been produced by using interesterification. Simple triglycerides, *i.e.* trioelin, trielaidin and tristearin, were used in the randomized interesterification reactions. The interesterified glycerides have been controlled analytically with high performance liquid chromatography. The polymorphic behaviour of the interesterified glycerides has been followed by recording of the diffraction pattern *versus* temperature. The result is compared with the polymorphic behaviour of the component triglycerides.

INTRODUCTION

The polymorphic behaviour of fats is important in many food systems, *i.e.* margarine and chocolate production. Information about the polymorphic forms and their transitions is therefore valuable.

Molecular arrangement in the α and the β , forms were recently proposed (1) which completes the earlier known structure of the β -form of simple triglycerides (2,3). Since the melt plays an important role when a fat crystallizes a structure of the melt was recently proposed (4).

Most studies of the polymorphic behaviour have been done with simple triglycerides, well defined triglycerides or binary mixtures. When a fat crystallizes, usually a fraction of the fat dictates the overall crystallization. Since most technical fats

consist of many different triglycerides, due to variation in chain length and saturation, the problem is often to understand which particular triglyceride fraction dominates the polymorphic behaviour.

To understand the polymorphic behaviour of a technical fat, both advanced analyses of the triglyceride composition and separate studies of each triglyceride fraction present are needed.

The present work deals with the possibility to study the polymorphic behaviour of complex glycerides formed by interesterification of simple triglycerides. The result is compared with the corresponding pure triglyceride systems.

The interesterification reaction of triglycerides has been reviewed in several articles (5-7). Unless the reaction is directed, for instance by crystallization, the acyl radicals are distributed in the new glycerides in a statistically random manner (5,6). It is thus possible to obtain well defined systems of triglycerides where the amount of each new glyceride is determined by the composition of the starting triglycerides.

We have used the randomized interesterification reaction to prepare systems of triglycerides containing stearic, oleic and elaidic acyl groups. The starting mixtures of triglycerides were chosen to produce glyceride systems that could serve as models for vegetable oils used in margarines.

The interesterified glycerides were investigated by means of high performance liquid chromatography, HPLC. For this specific purpose it was necessary to be able to quantify the amounts of different triglyceride species formed in the reaction in order to confirm that the distribution was equal to the one theoretically expected.

HPLC of triglycerides has been reported in the literature since the midseventies. The earlier works utilized in most cases the RI detector (8,9) but later also the UV detector was introduced in combination with solvent systems possible to use at short wavelengths (10). For the present application both detection principles were used in combination with a solvent system especially developed to be able to elute the higher saturated triglycerides such as tristearin (11) which in many of the reported HPLC systems cause trouble to detect and quantify.

The composition of oils used when producing margarine dominates of C_{18} hydrocarbon chains, stearin, olein and elaidin, *i.e.* a hydrogenated rapeseed oil with low content of erucic acid, LOBRA, consists of about 90% C_{18} chains (12). This homogeneity in the chain length gives a very rapid transition from the β' form to the β form which develops large crystals and ends in a margarine with unacceptable texture (it produces a sandy feeling in the mouth, cf. ref. 13). Possibilities to hinder the $\beta' \rightarrow \beta$ transition by using additives, like sorbitan stearate (14) and diglycerides (12,15) have been reported.

The possibility of using the variation in chain length to hinder the $\beta' \rightarrow \beta$ transition is also shown in the present work.

EXPERIMENTALS

Materials

The samples of 2-oleo-distearin (StOSt) and rac-1-oleo-distearin (OStSt) were prepared by standard methods. Triolein (OOO), trielaidin (E1E1E1) and tristearin (StStSt) were purchased from Sigma. Commercially available sodium methoxide was used as catalyst. In some reactions, lithium methoxide, prepared from methanol and butyllithium, was used.

Methods

Interesterification (16,17)

0.68 mmol of the triglyceride AAA (see Table 1) and 0.45 mmol of the triglyceride BBB were molten together in a reaction flask under a slow stream of nitrogen. About 5 mg of the catalyst (NaOMe or LiOMe) was added with stirring. The reaction mixture was stirred at 100°C for 4 hours. After cooling, 25-30 ml of methylene chloride was added and the organic layer was washed with three portions of water. After drying with sodium sulphate, the solution was filtered through a short column of neutral alumina. The triglyceride fraction was evaporated to yield about 800 mg (65-88%) of TLC-pure product.

HPLC

HPLC was carried out on the following equipment: LDC Minipump 711 with pulse dampener; Rheodyne loop injector Model 7125 ; 250 mm column 4.6 mm ID; Nucleosil 5 μ m C-18 stationary phase (Macherey-Nagel). The column was thermostated at 22°C. 200 μ g of the triglyceride mixture was injected in 10 μ l of hexane:isopropanol (1:1 v/v).

The solvent system was a ternary mixture consisting of acetonitrile, ethanol and hexane (40:35:25 v/v/v); all solvents were of spectroscopic grade.

The detection was performed with a Hewlett-Packard 1040 UV detector at 220 nm and an Altex Model 156 refractive index detector.

The chromatograms were recorded and integrated on a Shimadzy C-R2AX system.

X-Ray Diffraction

A diffraction pattern *versus* temperature (DPT) camera (18) was used. The X-ray films were examined with a photodensitometer. Immediately prior to the diffraction studies all samples were heated in the same way, *i.e.* heating for three minutes to a temperature of 30°C above the β -melting point, of the highest melting triglyceride fraction present, in order to destroy any "memory" of earlier crystals, and after that cooling to the temperature desired.

RESULTS AND DISCUSSION

The different pairs of triglycerides used and their molar ratio are listed in Table 1. The molar ratio 3/2 was chosen in order to give a solid-liquid ratio for the triolein-tristearin and the triolein-trielaidin systems similar to fats used in the margarine process.

The three investigated systems were each separated into the expected four peaks on the described HPLC system. The k' values and the equivalent carbon numbers (ECN) are reported in Table 2. k' Values are the normal way of expressing the retention behaviour of eluates in a HPLC system (cf. Table 2). The carbon number of a saturated triglyceride is defined as the number of carbon atoms in its fatty acids, *i.e.* tristearin $3 \times 18 = 54$. The ECN value of a triglyceride peak in HPLC is its experimentally found carbon number, calculated from a previous run of a set of saturated defined triglyceride species (9). The chromatogram from one of the systems (trielaidin - tristearin) is shown in Fig. 1.

The separated triglyceride types all have the same nominal carbon numbers (54) but different degree of unsaturation and configuration of the double bonds. This is the reason for using the ECN values, which give the chromatographic relation to the saturated triglycerides.

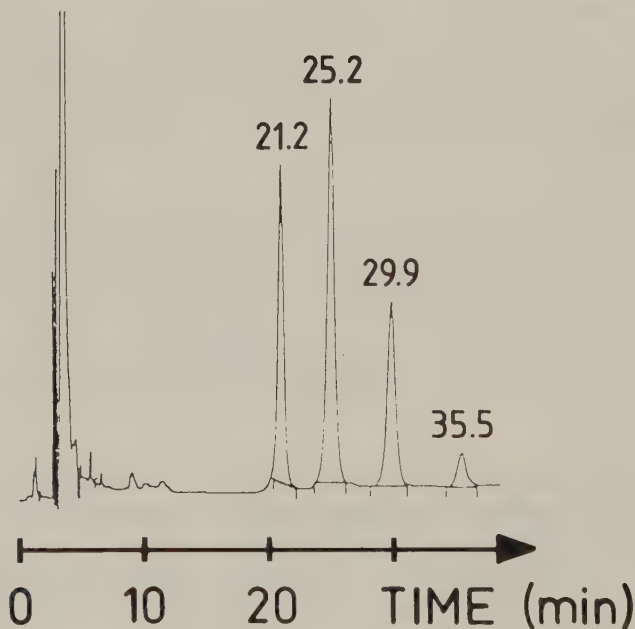


Fig. 1 HPLC of an interesterification system consisting of trielaidin and tristearin in the molar proportions 3:2. Chromatographic conditions are as described in the text.

Table 1. Starting triglycerides in the interesterification reaction.

Starting triglycerides		Molar ratios
AAA	BBB	AAA/BBB
OOO	StStSt	3/2
EIEIEI	StStSt	3/2
OOO	EIEIEI	3/2

OOO = triolein

EIEIEI = trielaidin

StStSt = tristearin

Table 2. ECN and k' values of the individual HPLC peaks from the three interesterification mixtures.

Triglyceride type		Experimental values	
Structure	Configuration	ECN	k'
OOO	54:3 (c,c,c)	46.8	3.9
O ₂ El	54:3 (c,c,t)	47.3	4.1
OEl ₂	54:3 (c,t,t)	47.8	4.3
EIEIEI	54:3 (t,t,t)	48.2	4.5
O ₂ St	54:2 (c,c)	49.1	4.8
El ₂ St	54:2 (t,t)	50.0	5.3
OSt ₂	54:1 (c)	51.4	6.2
ElSt ₂	54:1 (t)	51.9	6.5
StStSt	54:0	54	7.8

O = oleic

El = elaidic

St = stearic

ECN = equivalent carbon number

k' = capacity ratio; from $(t_r - t_0)/t_0$ where t_r is the retention time and t_0 the retention time of an unretained peak

54:3 (c,t,t) indicates one *cis* and two *trans* unsaturations

The quantitative results are reported as area % in Table 3. The general observation from these values is that the unsaturated triglycerides are overrepresented when the UV detection is used, compared to the RI-detection. This is valid even if the wavelength is 220 nm. This is observed in the two systems containing both saturated and unsaturated triglycerides, *i.e.* tristearin-triolein and tristearin-trielaidin, but not observed in the system with only unsaturated compounds (triolein-trielaidin). In the last case similar representations for the UV and the RI detectors are observed.

The general conclusion from the observed distributions is that the transesterification reaction has produced in each case a randomized mixture which corresponds well to the calculated composition. This has been demonstrated in the case of saturated triglycerides (17) and the present investigation shows that there is no selectivity due to double bonds or their configurations.

Table 3. Experimental results from HPLC determinations of interesterified systems of monoacid triglyceride pairs in the molar ratios 3/2.

Triglyceride type		AAA	A ₂ B	AB ₂	BBB
Theoretical distribution (mol %)		21.6	43.2	28.8	6.4
Found distribution (area %)					
OOO/EIEIEI	RI	21.9	44.7	27.6	5.8
	UV	20.2	42.3	30.2	7.3
OOO/StStSt	RI	23.2	43.8	26.7	6.3
	UV	32.3	41.0	22.6	4.1
EIEIEI/StStSt	RI	24.4	40.4	26.8	8.4
	UV	28.1	43.0	24.1	4.8

OOO = triolein (always A)
 EIEIEI = trielaidin
 StStSt = tristearin (always B)
 RI = Refractive index detection
 UV = UV detection

In Fig. 2 the polymorphic transitions of the interesterified triolein-tristearin mixture is shown. The mixture is cooled rapidly (80°C/min) down to 0°C and the DPT diagram is then developed during slow heating (0.4°C/min). Due to the rapid cooling the mixture crystallizes in the α -form and is then transformed to the sub- α -form on further cooling. The sub- α -form shows an extra line at 3.8 Å which indicates that the hydrocarbon chains are packed according to the orthorhombic subcell. When the temperature is raised the sub- $\alpha \rightarrow \alpha \rightarrow \beta' \rightarrow \beta$ transitions occur until the mixture finally melts at 56°C.

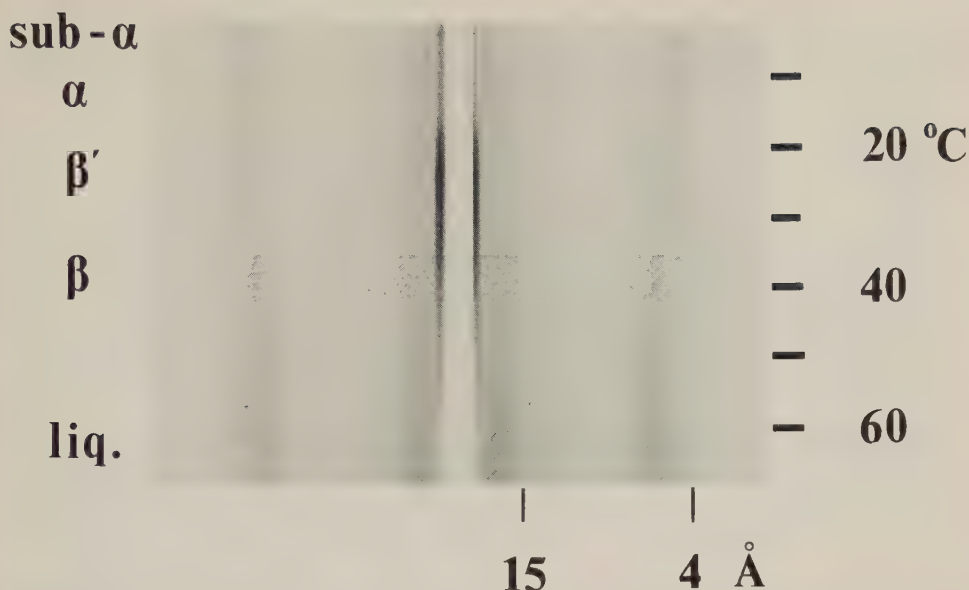


Fig. 2 DPT-diagram of the polymorphic transitions of an interesterified triolein-tristearin system, molar proportions 3:2. The mixture is cooled rapidly ($80^{\circ}\text{C}/\text{min}$) down to 0°C and the DPTdiagram is then developed during slow heating ($0.4^{\circ}\text{C}/\text{min}$).

The different triglycerides present in the interesterified tristearin-triolein system and their theoretical molar proportions are: tristearin (StStSt) 6.4, 2-oleo-distearin (StOSt) 9.6, rac-1-oleo-distearin (OStSt) 19.2, rac-1-stearo-diolein (StOO) 28.8, 2-stearo-diolein (OStO) 14.4 and triolein (OOO) 21.6. The theoretical and the analysed composition is shown in Table 3. In this table the oleo-distearins and the stearo-dioleins, respectively, are not separated, *i.e.* $A_2B = 43.2$ is equal to $(\text{StOO} + \text{OStO}) = (28.8 + 14.4 = 43.2)$. Examination of the melting points of these triglycerides reveals that tristearin, 2-oleo-distearin and rac-1-oleo-distearin dominates the crystallization process when the mixture is cooled. These triglycerides are therefore examined, with DPT, both separately and blended, in order to understand the crystallization of the interesterified system.

The result of these DPT studies is summarized in Fig. 4 (Polymorphic transitions when slowly heated ($0.4^{\circ}\text{C}/\text{min}$) after rapid cooling ($80^{\circ}\text{C}/\text{min}$)).

The rapid cooling of StOSt gives a sub- α -form that is transformed to α and β before melting occurs at 36.5°C (see Figs. 3 and 4). The longspacing indicates a triple chain length order which is consistent with an earlier proposed structure (20) where the olein chain in the 2-position of the triglycerides is proposed to be segregated and packed in one layer. This triple chain length is persistent through all transitions. The continuous shift of the inner longspacing for the sub- α and α -forms reveals that the tilt of the hydrocarbon chains is continuously decreased

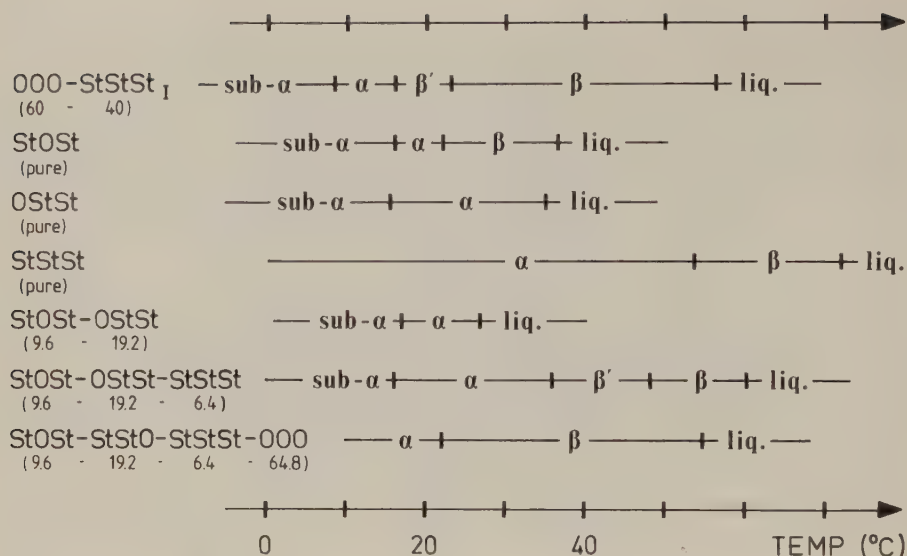


Fig. 3 Polymorphic transitions during slow heating (0.4°C/min) after rapid cooling (80°C/min) (based on DPT-diagrams as shown in Figs. 2 and 4).

I = Interesterified mixture.

Molar proportions are given in brackets.

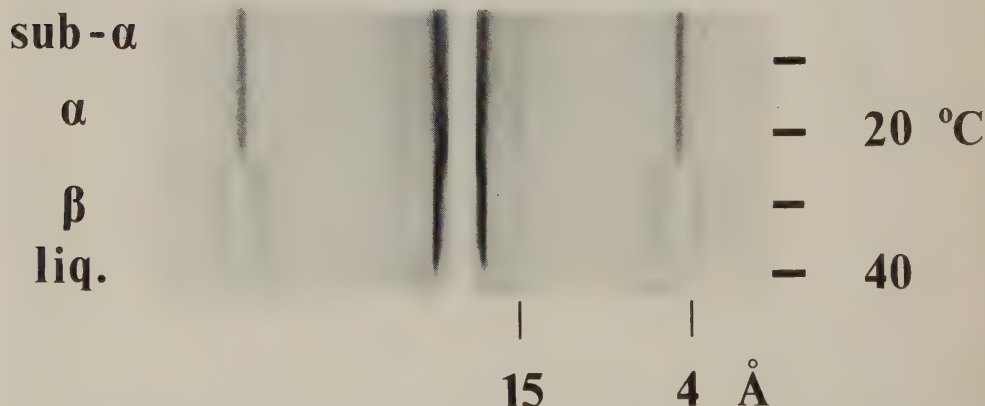


Fig. 4 DPT-diagram of the polymorphic transitions of 2-oleo-distearin. The sample is cooled rapidly (80°C/min) down to 0°C and the DPT-diagram is then developed during slow heating (0.4°C/min).

-- the d-value is shifted from about 38 Å at 0°C up to 46 Å when the temperature is 20°C. A similar behaviour has recently been shown for the α -form of 1-O-alkyl-glycerols (*cf.* Ref. 21).

The rapid cooling of OStSt gives a sub- α -form, which is transformed to the α -form at 15°C when the sample is slowly heated, and finally melts at 35°C. This triglyceride also shows triple chain length which perhaps is surprising since the unsaturated chains should be expected to be segregated in one layer if a triple chain length structure should exist. Hence the acyl chain in the 1-position in the triglyceride probably is oriented in the opposite direction to the others, contrary to the β -form of tridodecanoin, where the acyl chain in the 2-position is pointing in the opposite direction to the others (2).

The StOSt-StStO mixture crystallizes in a sub- α -form when the sample is cooled rapidly. When the mixture is heated the sub- $\alpha \rightarrow \alpha \rightarrow \text{liq.}$ transition takes place. Both the sub- α -form and the α -form is packed in a double chain layer hence the unsaturated chains are not segregated. The relative low melting point, 27°C, is due to the fact that the packing is not optimal.

The StOSt-StStO-StStSt mixture also crystallizes in a sub- α -form when cooled rapidly. The mixture undergoes the transitions sub- $\alpha \rightarrow \alpha \rightarrow \beta' \rightarrow \beta$ before melting finally occurs at 60°C. A continuous shift of the long-spacing lines for the sub- α -form when the temperature increases indicates that the tilt of the chain decreases. This change, however, is somewhat smaller compared with the one obtained in the case of the pure 2-oleo-distearin sample (see Fig. 3). The melting point of 60°C indicates that co-crystallization occurs. The intensity of the longspacing lines decreases continuously which must be due to variation in the homogeneity of the crystal packing. Regions containing more tristearin melt later than regions dominated of oleo-distearin.

The final step in imitating the triolein-tristearin interesterified system was to add 64.8 molar percent of triolein. By assuming that StOO and OStO do not influence the polymorphic behaviour this blend should be equal to the interesterified system.

This is, as can be seen in Fig. 4 not the case, the exclusion of both the sub- α and the β' -form indicates a difference. In the interesterified system in Fig. 2, the long-spacing for the sub- α and α -forms indicates the existence of a triple chain length order. However, when the $\alpha \rightarrow \beta'$ transition occurs the triple chain order disappears and in the β' - and β -forms only the double chain order exists. The StOSt-StStO-StStSt-OOO mixture gives double chain order and not triple in the α -form. Since the melting points for StOO and OStO fit with the disappearance of the triple chain length in the DPT diagram of the interesterified mixture in Fig. 2 these differences must be due to the OOST and OStO triglycerides present in the interesterified system.

2-Decano-diolein was recently proposed to give a β -3 structure (22) with the olein chains in the first and third positions in one layer.

The StOO and OStO glycerides can either crystallize segregated from the rest of the triglycerides in a triple chain length arrangement or co-crystallize with the rest of the triglycerides present. The latter possibility should give a segregation of the oleic acyl chains in one layer and the stearic acyl chains packed together with stearic acyl chains. This would give a triple chain order with saturated chains in two layers and unsaturated chains in the third one. This proposition is based on the fact that the α crystal forms are loosely packed, the mobility of the hydrocarbon chains is quite high, hence giving place for defects in the crystal packing.

When the temperature is raised the OStO and StOO glycerides melt, this happens at the same time as the $\alpha \rightarrow \beta'$ transition occurs (the better chain packing in the β' form excludes larger crystal deformations).

In order to study the influence of *trans* unsaturated fatty acids in a margarine oil blend, trielaidin was interesterified together with triolein and tristearin, respectively. The polymorphic behaviour of the triolein-trielaidin and the interesterified trielaidin-tristearin systems after rapid cooling (80°C/min) and then slowly heating (0.4°C/min), is shown in Fig. 5. In the same figure the polymorphic behaviour of a trielaidin-tristearin binary mixture, trielaidin and a binary mixture of the interesterified systems of triolein-tristearin and triolein-trielaidin with and without addition of 2-stearo-dipalmitin (PStP) is shown.

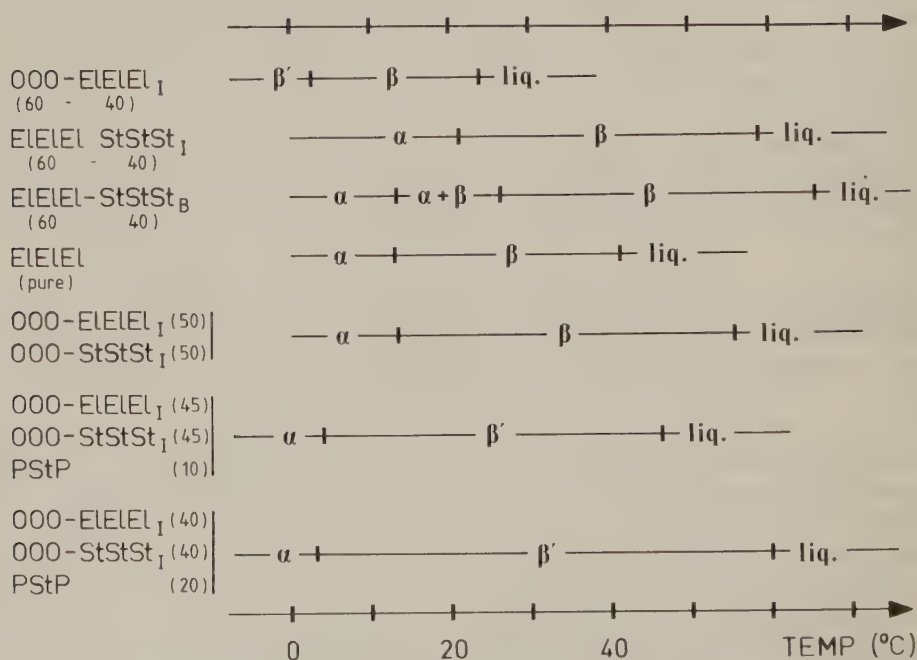


Fig. 5 Polymorphic transitions during slow heating (0.4°C/min) after rapid cooling (80°C/min) (based on DPT-diagrams as shown in Figs. 2 and 4).

I = Interesterified mixture

B = Binary mixture

Molar proportions are given in brackets.

The polymorphic behaviour of the interesterified triolein-trielaidin system shows similarities with the interesterified triolein-tristearin system. The main difference is the absence of sub- α and α in the case of the triolein-trielaidin system. However, due to the lower melting point of elaidin all transitions are shifted to a lower temperature. As a consequence the sub- α and the α -forms do not exist in the temperature region studied.

If the interesterified trielaidin-tristearin system, the trielaidin-tristearin binary mixture and trielaidin, see Fig. 5, are compared with tristearin in Fig 4, the presence of triglycerides containing elaidin in a triglyceride mixture can be somewhat clarified.

Trielaidin seems to behave as tristearin, both crystallize in the α -form which is transformed to the β -form before final melting. It should be mentioned that even if the β' -form is not detected in these studies this does not exclude the possibility that the transitions should be $\alpha \rightarrow \beta' \rightarrow \beta \rightarrow \text{liq}$. The only difference between trielaidin and tristearin is a temperature shift.

The melting point of the β -form of the interesterified EIEIEI-StStSt system is 58.5°C which is more or less in between the melting points of the pure samples. The polymorphic behaviour is identical, *i.e.* $\alpha \rightarrow \beta \rightarrow \text{liq}$.

The binary mixture of trielaidin and tristearin (see Fig. 5) has a different behaviour. The presence of a two phase region, *i.e.* $\alpha + \beta$, indicates segregated crystallization. However, the final melting point of the β -form at 65.5°C is much lower than the melting point of pure tristearin (72°C). Hence, the triglycerides are not totally segregated.

If the two interesterified systems of triolein-trielaidin and triolein-tristearin are blended in the ratio 1:1 the obtained mixture is quite similar to a hydrogenated fat. Both what concerns the content of *trans*-unsaturated fatty acids and the solid-liquid ratio.

In a hydrogenated fat the position of the double bond varies, which excludes the possibility to arrange the double bonds exactly as in the case of *i.e.* 2-oleo-distearin (20). Nevertheless the interesterified mixture is quite close to a hydrogenated fat. The main difference being the uniform chain length which is eliminated by introducing 2-stearo-dipalmitin.

Due to the homogeneity of the chain length the blend of the interesterified systems is transformed to the β -form before melting. The interesterified mixtures containing 2-stearo-dipalmitin melt in the β' -form. The more PStP the higher the melting point. Examination of the shortspacing lines of the β' -form of the mixture containing 20% 2-stearo-dipalmitin (3.84 m; 4.06; 4.24 m; 4.37 w) reveals that these are identical with the β' -form of pure 2-stearo-dipalmitin (cf. Ref. 1). This domination on the crystallization is due to the high amount and the high melting point of the 2-stearo-dipalmitin.

It should be pointed out that interesterification is fruitful not only when studying the polymorphic behaviour but also as a possibility to avoid *trans* unsaturated fatty acids. By total hydrogenation of a fat followed by interesterification with an actual oil, a fat with suitable crystallization properties could be produced. For nutritional reasons (23) the presence of *trans* fatty acids in fat products can thus be avoided.

ACKNOWLEDGEMENT

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IV

On Structural Relations between Lipid Mesophases and Isotropic Reversed Micellar (L2) Solutions

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The structural relations in lipid systems between liquid crystalline and isotropic liquid L2 phases have been studied by low-angle X-ray diffraction for two-model systems, viz. sodium octanoate/decan-1-ol/water and soybean lecithin/soybean oil/water. A transition between the two states may be obtained either by a change in temperature or in composition. The low-angle X-ray scattering curve for the liquid L2 solution shows a maximum with the same angular position as the innermost reflection given by a coexisting liquid crystalline phase, and the integrated intensities for the scattering maxima are almost the same. The relative amounts of ordered structural units are thus about the same on both sides of the transition. A close resemblance in the structures of the reversed micellar aggregates in the L2 solution and the liquid crystalline phase is thus proposed. If the liquid crystalline phase possesses a reversed hexagonal structure its long-range two-dimensional order will be replaced by a "medium" range order in the liquid phase but the latter will basically have the same hexagonal arrangement with the difference that the polar rod aggregates are short. If the liquid crystalline phase has a lamellar structure there will be a reduction in the size of the bilayer domains and an increase in their curvature.

INTRODUCTION

When the temperature of a lipid, with or without small amounts of water, is increased a series of liquid crystalline phases may be encountered, ultimately ending in an oily easily flowing isotropic liquid. A similar series of changes occurs when organic solvent is added. The resulting oily liquids where water is not the continuous medium are often termed L2 solutions.

In isothermal systems containing surface-active lipids (amphiphiles) and water one often encounters with increased lipid content the phase-transition sequence; aqueous non-micellar or micellar solution (L1) $\rightarrow$ liquid crystalline hexagonal "normal" phase (E) $\rightarrow$ liquid crystalline lamellar phase (D) (1). By adding an "oil" component this sequence will be extended. With increased oil content the phase structures become "reversed," and

one may find a second liquid crystalline hexagonal phase F before the isotropic solution phase (L2).

Proceeding from simple deliberations it is possible to show that such a transition sequence is a natural consequence of the volume requirements of the nonpolar and polar parts of the lipid molecules. In principle, these volume relations may be altered by two mechanisms (i) the internal mobility of the hydrocarbon chains is changed with the temperature and/or (ii) the extension of the hydrocarbon regions is increased by addition of an (liquid) apolar or weakly polar component. "Intermediate" phases interspersed between the above mentioned ones may also be found in many systems.

The present paper concerns the transition from liquid crystalline mesophases to isotropic "oil" phases. We have chosen as model systems the systems sodium octanoate/de-

can-1-ol/water and sunflower oil monoglyceride/soybean oil/water, and have studied the low-angle X-ray diffraction patterns for samples on both sides of the transition.

The isothermal phase diagram for the first system has been determined by Ekwall and co-workers (2). For high contents of decanol it shows the existence at 20°C of a reversed hexagonal phase (F) in equilibrium with a decanolic solution phase (L2) (Fig. 1) (2, 3). When the temperature is increased there is a transition $F \rightarrow L_2$; thus the region of existence of phase L2 will be increased (Fig. 2) (3, 4). At 40°C the L2 phase will be in equilibrium with the liquid crystalline phase D. The location of the border of the latter phase toward the two-phase region $D + L_2$ is not affected by a moderate further rise in the temperature (up to 80–100°C). While the internal structures of the liquid crystalline phases D and F of the model system are established, the detailed structure in the solution region L2 has been subject to many studies and much discussion. We believe that important

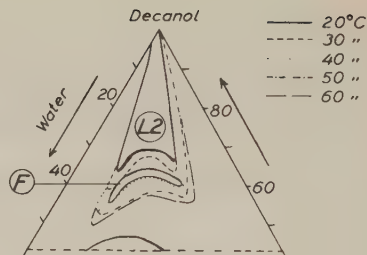


FIG. 2. The displacement of the boundary for the L2 region in the system sodium octanoate/decan-1-ol/water at temperatures between 20 and 60°C (3, 4). Notations as in Fig. 1.

conclusions about the structures can be obtained by the X-ray scattering technique. Samples from the L2-phase region, especially from the border toward the two-phase region $L_2 + F$, show pronounced diffuse X-ray scattering maxima in the low-angle region. Such a pronounced scattering is not observed for amphiphile substances in liquid state when the molecules are incapable of aggregation (5).

The ternary phase diagram for the system sunflower oil monoglycerides/soybean oil/water has recently been determined by Pilman *et al.* (6) (Fig. 3). A note on the structure of the L2 phase of a binary monoglyceride/water system has previously been published (5). In it is stressed the close relation, as revealed by the X-ray low-angle scattering phenomenon, between the lamellar liquid crystalline phase and the isotropic liquid L2 phase formed above the "melting point."

EXPERIMENTAL

Chemicals. The sodium soap was prepared by neutralization of octanoic acid (caprylic acid, purissimum, Fluka AG, Switzerland, or octanoic acid, specially pure, BDH, England) with sodium hydroxide (Titrisole, Merck AG, Germany). Some batches were obtained by neutralization with sodium ethoxide in absolute ethanol. The salt was recrystallized from absolute ethanol and dried in vacuum at 110°C. The molar mass was checked by titration with perchloric acid in glacial acetic

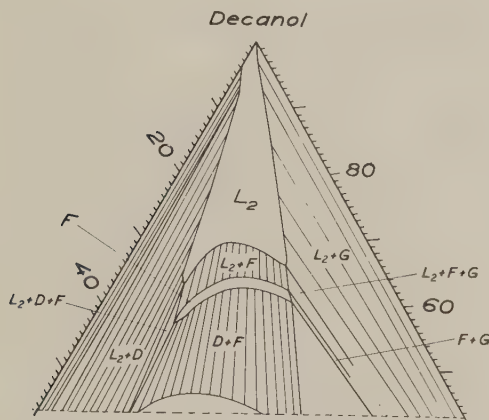


FIG. 1. Part of the isothermal phase diagram for the system sodium octanoate/decan-1-ol/water showing the extent of the solution region L2 at 20°C (redrawn after Ref. 3). Notations according to the work of the "Ekwall" school (2). L2, region for isotropic decanolic solution containing sodium octanoate and water. D and F, regions for liquid crystalline phases having a lamellar and a reversed hexagonal structure, respectively. G, region for solid crystalline substance. $L_2 + D$, $L_2 + D + F$, etc., two- and three-phase zones. Concentrations in (% w/w).

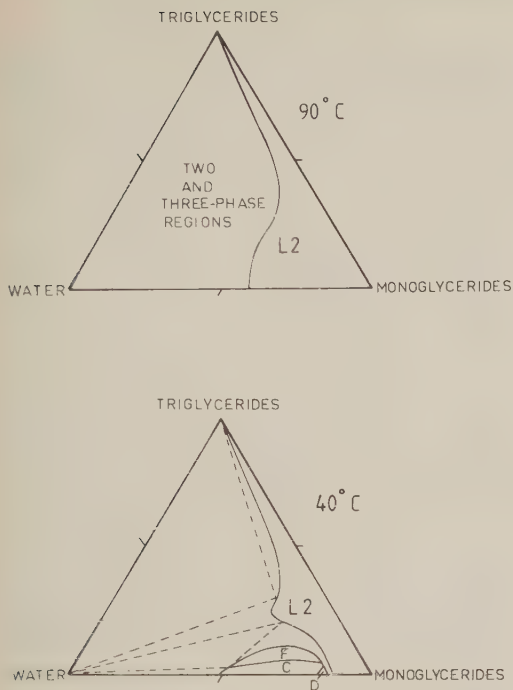


FIG. 3. The phase diagram for the system sunflower oil monoglyceride/soybean oil/water at 40 and 90°C. The two-phase regions and three-phase triangles are marked only at 40°C (6). Notations: L2, isotropic "oily" solutions; C, cubic liquid crystalline phase; D, lamellar liquid crystalline phase; F, reversed hexagonal liquid-crystalline phase. Concentrations in (% w/w).

acid, using crystal violet or oracetblue B as indicator, and differed less than 0.5% from the theoretical value. The decan-1-ol (Zur Zynthese, Merck AG, Germany) was vacuum-distilled and its water content was less than 0.0003 g cm^{-3} according to Karl Fisher titrations. (Decan-1-ol, specially pure, BDH, England, was used occasionally.) The water was double-distilled and ion-exchanged immediately before use. The sunflower oil monoglyceride and the soybean oil samples were of the same batches as used in another study (6).

Sample preparation. Samples were made by weighing appropriate amounts of the components in test tubes. These were flame-sealed and heated until equilibrium was attained. The samples were always stored at

least for a week at room temperature before the X-ray examination.

Apparatus. The X-ray diffraction pattern in the low-angle region was obtained with a Rigaku-Denki small-angle goniometer and the intensity of the diffractogram was measured by a scintillation detector and registered digitally. The copper K_{α} radiation was monochromatized by filtering through Ni foil. A camera designed to continuously record the diffraction pattern versus temperature (DPT) was also used (7). The highest spacing one could observe in this camera was about $50 \text{ \AA}$. Some diffractograms were obtained at fixed temperatures with a point-colimated Kiessig camera (8).

RESULTS AND DISCUSSION

The X-ray diffractograms from the liquid crystalline phases D and F of the sodium octanoate/decanol/water system contain in the low-angle region a series of sharp reflections, while samples from the isotropic liquid L2 phase give rise to a broad diffusion scattering maximum in the same angular region (4, 9, 10) (Fig. 4a). The diffractograms show in addition in the wide-angle region, both for liquid crystalline and isotropic liquid samples, a broad diffuse maximum at $4.5 \text{ \AA}$.

The scattering maximum in the low-angle region varies with composition for samples inside the L2-phase region both with regard to position and intensity. Pure decanol gives rise to a very weak scattering maximum with a position corresponding to about $16 \text{ \AA}$.¹ When the content of soap and water is increased the spacing corresponding to the position of this maximum increases to at most about $40 \text{ \AA}$ at the water-rich corner of the L2 region. The intensity and sharpness of the maximum (peak) is simultaneously strongly

¹ Such a weak scattering maximum as displayed by pure decanol is typical for liquid long-chain slightly polar compounds. An interpretation based on the radial distribution function indicates that its position for alcohols and fatty acids corresponds to the length of molecular dimers.

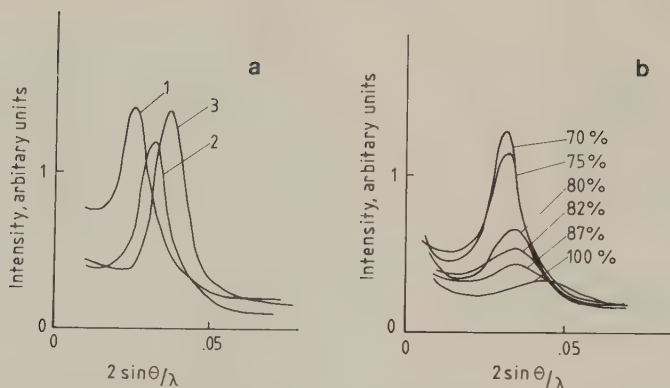


FIG. 4. The intensities of the X-ray scattering in the low-angle region for L2 samples in the system sodium octanoate/decan-1-ol/water (11). (a) At the border toward the two-phase region F + L2 molar ratio water/sodium octanoate is (1) 24.2, (2) 13.9, (3) 5.98. (b) For a descending series of samples from the decanol corner toward the sodium octanoate/water axis. The molar ratio water/sodium octanoate is 13.9 and the contents of decanol in the individual samples are 70, 75, 80, 82, 87, and 100%, respectively.

enhanced. This behavior is illustrated in Fig. 4b for a series of samples with different amounts of decanol and constant ratio in the amount of water to sodium octanoate ($n_{H_2O}/n_{NaC_8} = 13.9$).

Such a scattering phenomenon as shown by the L2 solutions is not consistent with the concept of a structure consisting of noninteracting aggregates with an aqueous core (water aggregates or inverted micelles). If this would be the case one should expect an X-ray scattering curve with a mainly Gaussian falloff.

One important factor of the experimental results for equilibrium samples is that the position of the scattering maximum given by the isotropic L2 solution coincides with that of the innermost reflection given by the liquid crystalline phase F (or phase D in other systems or at another temperature (Fig. 5) (Table I)). The same phenomenon is observed when the transition from liquid crystalline phase to isotropic solution is obtained by raising the temperature (Fig. 6).

Furthermore, it should be mentioned that our impression of a number of diffractograms from lipid/water systems is that the integrated intensity of the scattering maximum often is only a few percent lower for a L2 sample than of the diffraction peak for

the equilibrium liquid crystalline sample. This suggests that samples in equilibrium contain about the same relative amount of ordered structural units.

On the basis of these findings we suggest that the changes occurring at the transition $F \rightarrow L2$ in the system sodium octanoate/decanol/water depend upon a reduction in the size of the ordered domains. However, it should be noted that in the isotropic L2 so-

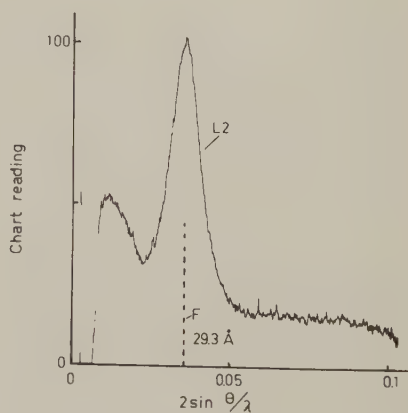


FIG. 5. The densitometer tracing of the X-ray scattering phenomenon in the low-angle region for the L2 part of a sample (composition 15/66/19, w/w) from the two-phase region F + L2 in the system sodium octanoate/decan-1-ol/water. The position of the maximum given by the equilibrium liquid-crystalline F part is marked by a broken line.

TABLE I

The Bragg Spacings Corresponding to the Maxima Given by L2 and F Phase Samples of Some Two-Phase Specimens in the System Sodium Octanoate (NaC_8)/Decan-1-ol (C_{10}OH)/water^a

Composition of the two-phase specimens, w/w			$\text{\AA}$	
NaC_8	C_{10}OH	H_2O	d in L2	d in F
10.0	62.0	28.0	42.9	39.3
10.0	65.0	25.0	40.2	37.9
11.0	67.0	22.0	33.7	34.8
13.0	68.0	19.0	31.5	31.1
18.0	67.0	15.0	28.1	28.1
21.0	65.0	14.0	27.0	27.4

^a Rigaku-Denki camera, 25°C.

lutions of this system a part of the decanol acts as a solvent while in the liquid crystalline hexagonal phase F the decanol molecules

(although not all) constitute an integral part of the aggregate entities (2).

It is thus proposed that the long-range two-dimensional crystalline order in the F phase is in equilibrium with a "medium-range" order in the L2 phase. The order in the latter phase, at least in the region close to the border toward the F phase, is basically the same hexagonal arrangement of rod-like aggregates only with the difference that the rods are short. The same conclusion has previously been drawn by Ekwall *et al.* in their evaluation of viscosity findings in the L2 region of the same and similar systems (12, 13).

The assumption of a transition from long-range order to medium-range order makes it natural to attempt an analysis of the line-broadening of the diffraction maximum obtained for L2 samples. A broadening of a diffraction line can be caused by variations

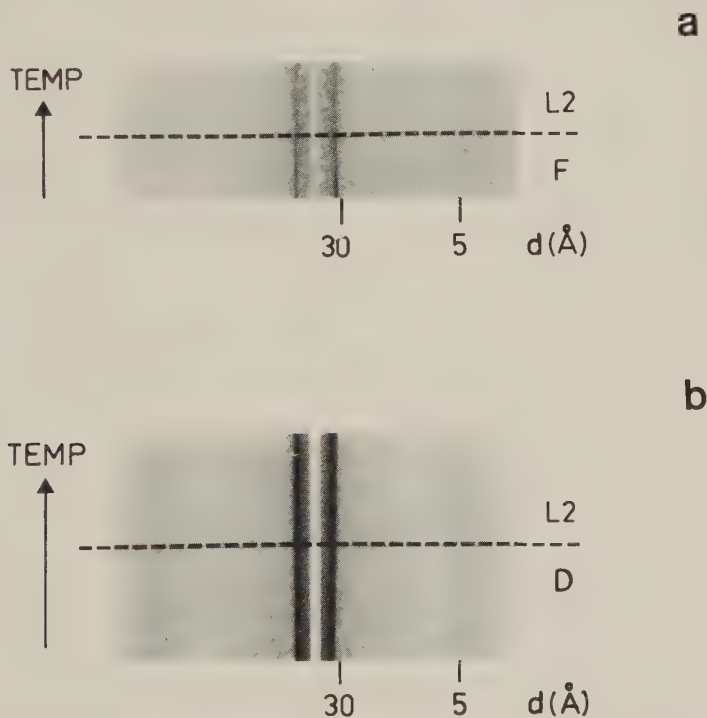


FIG. 6. DPT diffractograms showing the transition from liquid crystalline phase to isotropic L2 solution in the systems (a) sodium octanoate/decan-1-ol/water (sample composition 18.75/63.50/17.75, w/w; $\text{F} \rightarrow \text{L2}$, 20 $\rightarrow$ 60°C), (b) sunflower oil monoglyceride/soybean oil/water (sample composition, 82/3/15, w/w; $\text{D} \rightarrow \text{L2}$, 20 $\rightarrow$ 60°C). Note the coincidence in position of the reflection below and above the transition temperature, and that the main difference is an increased angular width of the peak.

in the size of the lattice parameters and/or in size of "single-crystal" domains (these should be smaller than about 10^{-5} cm). The Scherrer equation (14) for line broadening will in the present case have the form:

$$L_{11} = \frac{K \cdot \lambda}{\beta \cdot \cos \theta} \quad [1]$$

where L_{11} is the length of the crystallite domain perpendicular to the (11) plane of the hexagonal F-phase structure, K is a camera constant, β is the "pure" angular width of the diffraction peak at half-maximum intensity, θ is the diffraction angle, and λ is the wave length of the X-ray radiation (1.542 Å). In order to obtain the "pure" angular width for the L2 maximum we have, from the experimental value, subtracted the corresponding value for the F peak (assuming that the F phase consists of infinite rods, the width of its diffraction peak should be zero. The camera effects are thus included in the β value). In our setup the width of the F-phase diffraction peak was about 0.006 in the Kiessig camera, in the DPT camera the values were slightly higher 0.011–0.014, and for the L2-phase samples the widths of the maxima

were about 0.016 and 0.030, respectively (Table II). Under these conditions and assumptions we obtain $L_{(11)}$ values (corresponding to rod lengths) of 70–160 Å. (Due to the fact that the values for $\cos \theta$ are close to unity and shortcomings of the method the spread in the values is insignificant and only indicating that the values are in the range 100–200 Å.) Assuming the diameter of the rods to be the same as that for the aggregates of the F phase that would imply an axial ratio of 0.25 to 0.35. These ratio values are close to those deduced by Ekwall (12).

Parallel measurements in the system potassium octanoate/decanol/water gave similar values.

If the integrated intensity under a maximum is taken as a measure of the relative amount of the ordered structure, the findings for a series of samples in the L2 region with decreasing decanol content and constant soap/water ratio will indicate that the hexagonal arrangement of the rod aggregates become more and more ordered when the border toward the two-phase region L2 + F is approached (Fig. 4). This view of the structural relations between the F and L2 phases is further supported by our above-mentioned

TABLE II

Results from a Line-Broadening Analysis of Low-Angle X-Ray Scattering Maxima Obtained for L2 Samples in the System Sodium Octanoate (NaC_8)/Decan-1-ol (C_{10}OH)/Water

Composition (% w/w)			β_1 line width in F	β_2 line width in L2	$\Delta\beta$	$L_{(11)}$ (Å)
NaC ₈	C ₁₀ OH	H ₂ O				
A. Thermal transition (20 → 40°C), F → L2, DPT camera						
12.18	60.00	27.82	0.011	0.029	0.019	90
13.74	62.50	23.76	0.011	0.031	0.020	80
18.75	63.50	17.75	0.013	0.029	0.016	90
21.54	63.00	15.46	0.014	0.029	0.015	100
B. Isothermal equilibrium, F + L2, Kiessig camera, 20°C						
Composition of the two-phase samples (w/w)						
11.0	61.5	27.5	0.006	0.017	0.011	140
11.94	65.73	22.33	0.006	0.016	0.010	150
18.22	65.45	16.33	0.006	0.015	0.009	170

finding that the position of the intensity maximum in the diffractograms for L2-phase samples coincides with the position for the first-order peak for the F-phase samples when the samples are in equilibrium (Fig. 5).

An example of L2 solutions which are of interest in connection with food products is given by the ternary polar lipid/fat/water systems (6, 15). As has been mentioned above and demonstrated in a previous paper (5) the transition from liquid crystalline phase to L2 phase, when achieved by heating, is similar in nature to that examined above in the octanoate/decanol/water system. A DPT diffractogram of the transition from the D-phase structure to the L2 phase is shown in Fig. 6. The X-ray pattern changes at the transition from the lamellar liquid-crystalline phase to the L2 phase are similar to those described for the soap/long-chain alcohol/water systems. The integrated intensity is of the same magnitude below and above the transition temperature, and the positions of the intensity maxima coincide. The structure relations between the liquid crystalline phase and the L2 phase at a thermal transition are therefore proposed to be the same. An estimation of the size of the ordered domains, based on line broadening in the same manner as described above, results in values around 100 Å. The aggregate structures of a liquid crystalline phase and its neighboring isotropic solution phase are thus similar irrespective of whether the transition from one phase to another is obtained by a change in temperature or in composition. When the transition is from a hexagonal phase to a L2 solution the polar rod-like units with a water core covered with amphiphile molecules will become shorter whereas when the transition is from a lamellar phase there will be a reduction in size of the ordered lamellar domains and a change in the bilayer curvature.

There is a large group of isotropic surfactant solutions containing at the same time high amounts of water and oil. Such solutions are often termed "microemulsions,"

but the term has been given widely varying interpretations. With reference to the original definition by Schulman the microemulsions (transparent emulsions) are to be regarded as L2 solutions in which hydrocarbon has been dissolved (16, 17). One extreme boundary for microemulsion systems could therefore be the L2 solutions of ternary amphiphile systems and the addition of a hydrocarbon will successively reduce the order of the system. When the cosurfactant is a long-chain alcohol such as decanol the polar rod aggregates will remain closed but when it is pentanol or butanol there is no marked separation into distinct hydrophilic and hydrophobic domains separated by well-organized interfaces.

The L2 solutions will, in some systems, continuously merge with the aqueous micellar L1 solutions and there will thus be a transition zone where the structure presumably is bicontinuous. While some authors include the common L1 and L2 solutions in the notion "microemulsions" others require the presence of a hydrocarbon in the solutions as well. The problems of microemulsions are presently the subject of intense research (18–25). In a present note an attempt has been made to obtain a common manageable definition of the microemulsion concept (26). A comprehensive term for all the existing varying liquid isotropic systems containing water, oil, and amphiphilic lipids is certainly needed. However, there is at least one chief characteristic of microemulsions according to this definition—they are not emulsions.

Finally, it should be noted that Senatra (27) has reached similar conclusions using quite different techniques in a study of the transition from the "microemulsion" to the liquid crystalline phase in the quarternary system potassium oleate/hexan-1-ol/n-dodecane/water. She suggests that "a kind of latent liquid crystalline mesophase develops, not yet spatially organized . . ." when the "microemulsion" is in contact with the reverse hexagonal liquid-crystalline phase.

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Polymorphism of Rapeseed Oil with a Low Content of Erucic Acid and Possibilities to Stabilise the β' -Crystal Form in Fats

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The polymorphism of hydrogenated rapeseed oil with a low content of erucic acid was investigated by X-ray diffraction. The triglyceride composition in the hydrogenated oil was determined by a combination of gas chromatography and high-performance liquid chromatography. A characteristic feature of this oil is its rapid transition to the β -form. Possibilities of influencing the $\beta' \rightarrow \beta$ transition are discussed. The addition of diglycerides appears to provide a means for delaying the $\beta' \rightarrow \beta$ transition. Thus, 1,2-diglycerides show a strong stabilising effect on the β' triglyceride crystals form, which is considered to be of technical interest.

1. Introduction

The content of erucic acid in rapeseed oil grown in Sweden has by plant breeding been reduced from about 50% down to about 1%. This change has given a very homogeneous fatty acid pattern, with C₁₈ acids constituting nearly 90% of the acyl groups (Table 1). A consequence of this different fatty acid composition is, however, undesired changes of the physical characteristics in the hydrogenated oil, particularly of the polymorphic behaviour. The earlier type of rapeseed oil crystallises with a relatively stable β' -form,^{1–4} whereas rapeseed oil with low content of erucic acid (LOBRA) has a strong tendency for rapid transitions into the β -form.^{5,6} A margarine produced by standard methods from LOBRA (after hardening) results in a product which is unacceptable due to its texture (it produces a sandy feeling in the mouth, cf. reference 7). The present work concerns the polymorphism of LOBRA, and one way of stabilising the β' -form which is considered to be of technical interest is reported. Other possibilities of achieving this effect have been reported,⁸ and a theoretical discussion of the mechanisms involved is presented.

In order to evaluate the composition of triglycerides in the hydrogenated LOBRA oil it was submitted to a combination of analyses. A complete determination of all individual triglycerides is not possible at the present time. However, the combination of a few analytical steps provides enough information to determine the specific *types* of triglycerides present in the oil.

2. Experimental

Fully refined and deodorised rapeseed oil of a low erucic acid variety was used. The oil was hydrogenated in a laboratory reactor with a commercial nickel catalyst to a melting point of 40°C. The final fatty acid composition, including the content of *trans* double bonds (AOCS method Cd 14-61) is shown in Table 1.

The polymorphism was investigated by X-ray diffraction data in order to identify the triglyceride crystal forms.⁹ DPT diagrams (diffraction pattern *vs* temperature recording) were used according to Stenhagen,¹⁰ and X-ray powder data were also recorded using a Guinier-type of focusing camera.

In order to study the crystallisation effects, the samples were treated in the following way. To produce homogeneous samples they were held at 70°C for 2 h. An interesting observation was that

Table 1. Fatty acid composition of hydrogenated LOBRA 40

Fatty acid	Weight (g kg ⁻¹)
16:0	56
18:0	288
18:1	616
18:2	18
20:0	10
20:1	8
22:0	4

Trans content = 666 g kg⁻¹ (AOCS method CD 14-61).

Components < 2 g kg⁻¹ not included.

when the samples contained diglycerides, little or no acyl migration of the diglycerides occurred. The order¹¹ of the triglycerides in the liquid state obviously immobilises the diglycerides and inhibits acyl migration. Immediately prior to the crystallisation experiments all samples were treated in the same way—80°C for about 15 min, in order to destroy any 'memory' of earlier crystals, and after that cooled, usually at 10°C min⁻¹, to the particular temperature.

A combination of gas chromatography (g.c.) and high-performance liquid chromatography (h.p.l.c.) was used as described in Figure 1. The LOBRA oil was separated according to partition numbers and collected as fractions. The oil and these fractions were submitted to two different g.c.-analyses: intact triglycerides (separation according to 'carbon numbers') and fatty acid distribution (transformation to fatty acid methyl esters). From this the amount of triglyceride was calculated, expressed as the carbon number with a defined degree of unsaturation, e.g. 52:2, 54:3, etc. In this case the fatty acid distribution only gave possible combinations which fitted these calculations but definitely excluded others, and therefore it was not considered further for this purpose.

Analytical h.p.l.c. was carried out on a 250 mm column (4.6 mm i.d.) packed with 5 µm C 18 spherical particles (Nucleosil, Macherey-Nagel). Semi-preparative h.p.l.c. was performed on a 250 mm column (10 mm i.d.) packed with 5 µm C 18 irregular shaped particles (Polygosil, Macherey-Nagel). The eluent mixture consisted of methanol-acetone (60:40). The rest of the h.p.l.c. equipment consisted of an LDC Minipump 711 with pulse damper, a Rheodyne 7120 loop injector and refractive index detectors (LDC Refractomonitor and Optilab Multiref).

In the semi-preparative case 20 mg of original oil was injected each time with 100 µl of chloroform. Collected fractions from several injections were combined and submitted to further analyses.

G.c. of intact triglycerides was carried out on a Varian 1440 gas chromatograph equipped with an FI-detector. The 50 cm (2 mm i.d.) glass column was packed with 3% OV-1 on Gas-chrom Q (100–120 mesh) and run at a temperature programme from 200 to 350°C (2°C min⁻¹).

The original sample and the triglyceride fractions were transformed to the corresponding fatty acid methyl esters with sodium methoxide in methanol. The g.c. analysis was performed on a 200 cm (2 mm i.d.) glass column packed with 6% BDS on Anachrom ABS (100–110 mesh) isothermally at 180°C.

3. Results and discussion

3.1. Glyceride structure of LOBRA rapeseed oil

The results from the triglyceride analyses of the hydrogenated rapeseed oil, LOBRA 40 (i.e. hydrogenated to a melting point of 40°C), are shown in Table 2. Four types are responsible for > 80% (52:2, 54:1, 54:2, 54:3) and two of these (54:2, 54:3) represent 70% of the triglycerides. It is an interesting observation that only a small amount of trisaturated triglycerides was formed during the hydrogenation process. The original LOBRA oil has been investigated by the same procedure, and a comparison is given in Table 3 between the original and the hydrogenated oil regarding the triglyceride types which represent > 5% of the original oil.

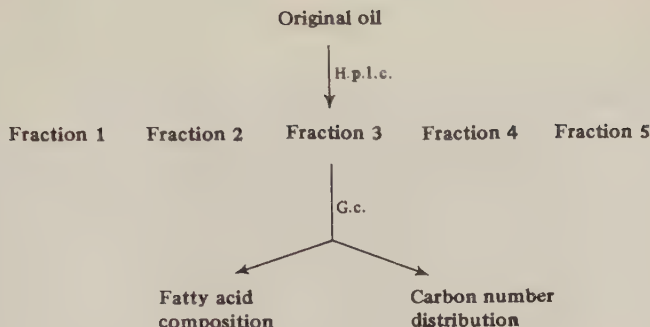


Figure 1. Schematic representation of the analysis of the hydrogenated rapeseed oil.

Table 2. Distribution of triglyceride types in hydrogenated LOBRA 40

Saturated (mol %)		Mono-unsaturated (mol %)		Di-unsaturated (mol %)		Tri-unsaturated (mol %)		Tetra-unsaturated (mol %)	
50:0	0.1	48:1	Traces	50:2	0.1	52:3	0.9	54:4	3.6
52:0	0.5	50:1	0.3	52:2	8.8	54:3	40.1		
		52:1	3.7	54:2	29.3	56:3	2.7		
		54:1	6.0	56:2	2.4	58:3	0.2		
		56:1	0.3	58:2	0.7	60:3	0.3		
Total	0.6		10.3		41.3		44.2		3.6

Table 3. Comparison of the most frequent triglyceride types in the original rapeseed oil with corresponding types from the hydrogenated oil

Type	Original oil (mol %)	Hydrogenated oil (mol %)
54:3	26	40
54:4	22	4
54:5	23	—
54:6	8	—

3.2. Polymorphic behaviour of hydrogenated LOBRA

A DPT diagram of hydrogenated LOBRA 40 is shown in Figure 2. The conditions were as follows: sample temperature 70°C for 15 min, followed by cooling for about 4 min to 23°C. It should be noted that a temperature of 80°C for 15 min (as the handling of the other samples corresponds to Table 4) gave the same transition rates; as evident from the X-ray diffraction diagrams. It is noticeable that the time the α -form exists is very short, in fact the $\alpha \rightarrow \beta'$ transition starts within 2 min and is completed after < 5 min. The $\beta' \rightarrow \beta$ transition starts within 3 h and after about 10 h practically

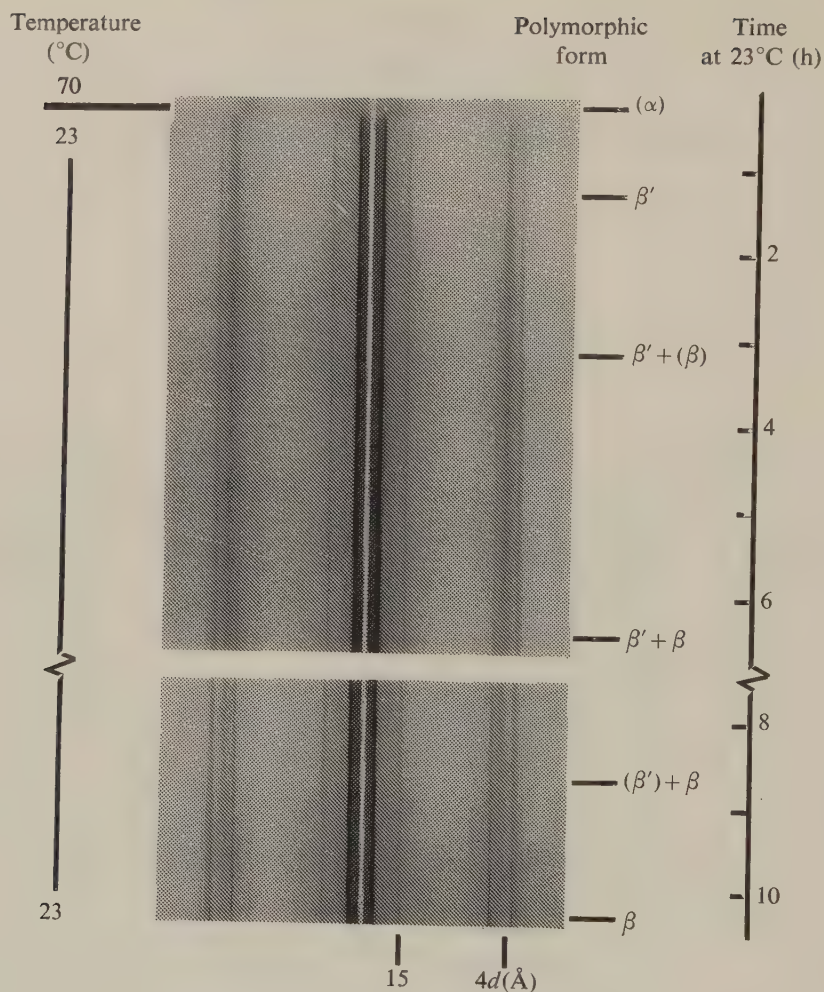


Figure 2. DPT diagram of hydrogenated LOBRA 40.

Table 4. Influence of dipalmitin on the $\beta' \rightarrow \beta$ transition of hydrogenated LOBRA 40

Hydrogenated LOBRA 40 (g kg ⁻¹)	Sample		Storage time at 23°C (days)		
	rac-1,2-PP ^a (g kg ⁻¹)	1,3-PP ^a (g kg ⁻¹)	1	3	7
975	25	—	β'	β'	β
950	50	—	β'	β'	β
925	75	—	β'	β'	β
975	—	25	β'	$\beta' + (\beta)$	β
950	—	50	β'	$(\beta') + \beta$	β
925	—	75	$\beta' + \beta$	β	β
925	50	25	β'	β'	β
900	50	50	β'	β'	β
875	50	75	β'	$\beta' + (\beta)$	β

^a1,2-PP and 1,3-PP = 1,2- and 1,3-dipalmitoyl glycerol, respectively.

all crystals are transformed to the β -form. In Figure 2 the extent of each polymorphic form is shown.

If hydrogenated LOBRA 40 is blended with 5% of rac-1,2-dipalmitoyl glycerol (rac-1,2-PP) and treated in the same way as the pure hydrogenated LOBRA 40 (described earlier) an interesting prolongation of the α -life time to about 20 min is obtained. 1,3-Dipalmitoyl glycerol (1,3-PP) does not have this effect. Dorset and Pangborn¹² have recently studied the polymorphism of diglycerides. 1,2-Diglycerides are known to crystallise in a β' -form with orthorhombic chain packing, whereas 1,3-diglycerides crystallise in a β -form with triclinic chain packing. It should be expected that all diglycerides, like mono- and triglycerides, have to pass through an α -crystal form with hexagonal chain packing when the melt solidifies. The prolongation of the α -life time when LOBRA 40 is blended with rac-1,2-PP indicates: (i) diglycerides are able to co-crystallise with triglycerides; and (ii) diglycerides are able to influence the polymorphic behaviour of a triglyceride mixture.

3.3. Possibilities of influencing the $\beta' \rightarrow \beta$ transition

Two general types of β' -structure stabilisation may be expected to delay the $\beta' \rightarrow \beta$ transition: (1) stabilisation of the orthorhombic chain packing; and (2) variation of chain lengths so that there is a disordered chain penetration region near the methyl and group planes.

The first of these alternatives requires mixed crystal formation of a substance which is better accommodated into crystals with an orthorhombic chain packing than in crystals with triclinic chain packing. Diglycerides are of particular interest in this respect, as they are known to be organised in an extended form,¹² i.e. they should be expected to fit a triglyceride bilayer and thus tend to form mixed crystals. As will be demonstrated 1,2-diglycerides have a strong stabilising effect on the β' triglyceride crystal form. The prolongation of the α -life time in the case of rac-1,2-PP, as noted earlier, indicates that mixed crystals are formed.

The second proposed alternative to delay the $\beta' \rightarrow \beta$ transition by 'disturbing' the crystal lattice, when a mixed crystal is formed of triglycerides with different chain lengths compared with LOBRA, is based on the following argument. Variations in triglyceride chain lengths must result in a crystal packing where a zone near the methyl and group planes is highly disordered. There is crystal structure evidence for the existence of disordered hydrocarbon chains near the methyl end groups. Thus potassium caprate¹³ has been reported to exhibit an A $\rightarrow$ C transition at 76°C, where the unit cell dimensions are almost unchanged beside an elongation in the long-spacing direction, suggesting a partial 'melting' of the chains near the methyl end groups. If there is a high degree of disorder in the methyl end group region, it should be expected that the energy difference between the β' and β -crystals form will be reduced, and therefore also the driving force towards a $\beta' \rightarrow \beta$ transition. Chain penetration in the disordered regions between adjacent layers might provide further hindrance towards the transition.

With regard to both effects based on mixed crystal formation, the kinetics of the crystallisation will be important for a consideration of the technical effects (cf. reference 14).

The strong stabilising effects of 1,2-diglycerides on the β' -triglyceride crystal form is shown in Table 4. The same nomenclature is used in Table 4 and Figure 2. It is obvious that diglycerides are able to delay the $\beta' \rightarrow \beta$ transition, for comparison see Figure 2, where the pure LOBRA 40 begins to develop the β -form within 3 h at 23°C. As noted earlier, 1,2-diglycerides prefer the orthorhombic chain packing and 1,3-diglyceride the triclinic one. This is the reason why 1,2-diglycerides stabilise the orthorhombic β' -form of triglycerides to a greater extent than 1,3-diglycerides. The stabilising effect of 1,2-diglycerides does not seem to be hindered by 1,3-diglycerides, at least not if the amount of the latter is kept within reasonable limits. The amount of 1,2-diglycerides can be kept quite low, in fact only 1% is needed to delay $\beta' \rightarrow \beta$ transition. The effect of 1,3-diglycerides on the β' stability in palm oil has been reported previously,¹⁵ and the results are consistent with the effects described here.

One interesting observation is that the chain packing of glycerides depends on which optical form is present. Thus, it seems to be the sn-2,3 form that delays the $\beta' \rightarrow \beta$ transition more than the sn-1,2 isomer.

The effect of different chain lengths of the added diglycerides on the stabilising effect is very

pronounced. Since hydrogenated LOBRA contains practically nothing but C-18 chains (Table 1) it is not surprising that distearin can delay the $\beta' \rightarrow \beta$ transition even more than dipalmitin. Experiments performed with dieicosanoin showed a shorter delay in the $\beta' \rightarrow \beta$ transition than with the addition of dipalmitin. Thus, it seems that the maximal delay of the $\beta' \rightarrow \beta$ transition is obtained if the chain lengths of the added diglycerides are the same, or very similar, as the chain lengths of the triglycerides. The shorter chain length of the diglycerides, the smaller is the possibility to stabilise the orthorhombic form. If the chains are too long, it is probable that problems with co-crystallisation will occur.

Blending LOBRA with diglycerides as a technical possibility for producing margarine will be studied in a pilot-plant evaluation. It should be emphasised that from a nutritional point of view diglyceride is equivalent to a fat, and the advantage of diglycerides compared with additives such as sorbitan tristearate (used for β' stabilisation⁸) are obvious. The possibility of using diglyceride to inhibit blooming in chocolate products might also be of technical interest.

The effect of a variation of chain length in triglycerides will be studied as a continuation of the present work using a model system containing well-defined triglycerides that correspond to LOBRA. By adding pure components to change the triglyceride composition it might be possible to predict the polymorphic behaviour of other triglyceride mixtures. This information could be used when producing margarine, and in plant breeding as a guide to the role of the triglyceride structure in relation to crystallisation properties.

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VI

Diglycerides as a Stabilizer of the β' -Crystal Form in Margarine and Fats

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The possibility of using diglycerides as a β' -stabilizer in fats was studied both in lab-scale and in pilot plant equipment. The margarine studied was of table type and contained a high level of hydrogenated rapeseed oil with low content of erucic acid, LOBRA. A characteristic feature of this fat is its rapid transition to the stable β -form. After producing margarines with different amount of diglycerides the polymorphic transitions were followed by X-ray diffraction during storage. The results clearly show the potential of using diglycerides as a β' -stabilizer, which must be of technical interest.

Introduction

The content of erucic acid in rapeseed oil grown in Sweden has by plant breeding been reduced from about 50% down to about 1%. This has given a very homogeneous fatty acid pattern, with C_{18} acids constituting about 90% of the acyl groups (Table 1). A consequence of this different fatty acid composition is, however, undesired changes of the physical characteristics in the hydrogenated oil, particularly of the polymorphic behaviour. The earlier type of hydrogenated rapeseed oil crystallizes with a relatively stable β' -form¹, whereas hydrogenated rapeseed oil with low content of erucic acid (LOBRA) has a strong tendency for rapid transitions into the β -form^{2,4}. A margarine produced by standard methods with hydrogenated LOBRA in the recipe may, upon storage, change to a product which is unacceptable due to its texture (it produces a sandy feeling in the mouth, cf. Ref.⁵).

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Diglyceride als Stabilisator der β' -Kristallform in Margarine und Fetten

Die Möglichkeit, Diglyceride als β' -Stabilisator in Fetten einzusetzen, wurde sowohl im Laboratorium als auch in größeren Versuchsanlagen studiert. Die hierfür verwandte Margarine war eine tafelfertige Sorte und enthielt hydriertes Rapsöl mit niedrigem Erucasäure-Gehalt, genannt LOBRA. Eine charakteristische Eigenschaft dieses Fettes ist sein schneller Übergang in die stabile β -Form. Nach der Herstellung verschiedener Margarine-Sorten mit unterschiedlichen Diglycerid-Mengen verfolgte man die polymorphen Übergänge während der Lagerung mit Hilfe der Röntgen-Diffraktion. Die Ergebnisse zeigen deutlich, welche Möglichkeiten die Verwendung von Diglyceriden als β' -Stabilisator bietet, was von gewissem technischen Interesse sein dürfte.

Table 1

Fatty acid composition of the used oils, percent by weight

Fatty acid	LOBRA 34	LOBRA 40	Soya oil	Soya 41
16:0	3.9	4.1	10.7	10.8
16:1	0.5	0.3	—	—
18:0	14.0	24.0	3.5	19.4
18:1	69.3	62.6	21.9	64.3
18:2	7.2	3.5	54.9	4.3
18:3	0.3	—	7.8	—
20:0	0.8	1.2	0.4	0.4
20:1	1.8	1.9	0.4	0.3
22:0	0.5	0.8	0.4	0.5
22:1	1.7	1.6	—	—

Trans content (%) 42 43 45
(AOCS method Cd 14-61)

Components < 0.2% not included

LOBRA = Rapeseed oil with low content of erucic acid

Diglycerides have been shown to delay the $\beta' \rightarrow \beta$ transition in hydrogenated LOBRA⁴. The present work concerns the stabilization of the β' -form with diglycerides in margarines based on hydrogenated LOBRA.

Materials and Methods

Fully refined and deodorized rapeseed and soybean oils were used. The rapeseed oil, a low erucic acid type, was hydrogenated to a melting point of 34 and 40°C (LOBRA 34 and LOBRA 40) and the soya oil was hydrogenated to a melting point of 41°C (Soya 41). The hydrogenation was carried out in full scale equipment using a commercial nickel

catalyst. The fatty acid composition of the different oils (determined with GLC), including the content of trans double bonds (AOCS method Cd 14-61) is shown in Table 1.

The diglyceride used in the experiments was obtained from Grindsted A/S, Brabrand, Denmark, as a byproduct when producing monoglycerides from lard. It was a technical grade containing approximately 68 % diglycerides, 26 % triglycerides and 6 % monoglycerides. ~ 60 % of the fatty acids were stearin and 25-30 % palmitin, ~ 60 % of the diglycerides were in the 1,3 form and ~ 40 % in the rac 1,2 form.

Solid fat content was determined by pulse NMR. The triglyceride crystal forms⁶ were identified by X-ray diffraction⁷. Both a homebuilt powder diffractometer and a Philips PW 1050 diffractometer were used.

The oil blend used in the experiments consisted of 30 % LOBRA 34, 20 % LOBRA 40 and 50 % unhydrogenated soya oil.

The lab-scale experiments were performed as follows. The oil blends were heated to 80° C and kept there for 15 min followed by rapid cooling down to 5° C whereafter the samples were stored at 21° C. The polymorphic transitions were then followed.

Margarines were produced on a Gerstenberg laboratory perfector 2-57-NH₃. The set up consisted of a cooling unit followed by working, cooling and packing units. The capacity was 50 kg/h and the margarine had an exit temperature of 5-6° C.

The margarine blends contained: 80 % oil blend, 9.8 % fermented skimmilk, 0.25 % lecithin, salt, aroma, colour and water. After production the margarines were stored at 20° C. Since sandiness is the negative effect of the β' to β transition a visual judgement of the margarines was made after 6 weeks beside the X-ray diffraction studies.

Since diglycerides delay the β' to β transitions when mixed with for instance hydrogenated LOBRA 40⁸ the oil blend mentioned above was mixed with different ratios of diglyceride, the recipes are shown in Table 2. The diglyceride

Table 2
Composition of the different oil blends, weight percent

	Recipe					
	1	2	3	4	5	6
Soya oil	50	50	50	50	50	50
LOBRA 34	30	30	30	30	30	30
LOBRA 40	20	19.5	19	18	15	15
Soya 41						5
Diglycerides		0.5	1	2	5	

was introduced at the cost of the LOBRA 40. In order to see if the reduction of LOBRA 40 gave any changed polymorphic behaviour, hydrogenated soya oil was introduced instead of diglycerides in recipe 6.

Results

The recipe used in the experiments gave a solid/liquid ratio about what is normal for a table type of margarine, see Table 3, recipe 1. Since the only solid fat present was hydrogenated LOBRA, the oil could be considered as pure LOBRA-based as far as concerns the polymorphic behaviour. It was

therefore expected to be rapidly transformed to the stable β -form upon storage.

The results from the lab-scale experiments are shown in Table 4. The β -form was fully developed after 4 days for re-

Table 3
Solid fat content for the different oil blends (%)

Temp. (°C)	Recipe					
	1	2	3	4	5	6
20	14.2	14.4	14.6	15.1	17.1	14.6
30	4.8	5.1	5.0	5.8	7.9	4.7
35	1.7	1.7	2.6	2.8	4.4	1.9
40	0	0	0	0	1.4	0

Table 4

Polymorphic forms present in the lab-scale oil blends, storage at 21° C

Recipe no.	Diglycerides (%)	3 days	5 days	10 days	14 days
1	0	(β') + β	β	β	β
2	0.5	(β') + β	β	β	β
3	1.0	β' + (β)	β' + (β)	β	β
4	2.0	β' + (β)	β' + (β)	β' + β	(β') + β
5	5.0	β' + (β)	β' + (β)	β' + β	(β') + β
6	0*	β' + β	β	β	β

* 5 % Soya 41 instead of diglycerides

Table 5
Relative tendency of β' to β transition in the pilot plant margarine on storage at 20° C

	Recipe					
	1	2	3	4	5	6
Diglycerides (%)	0	0.5	1	2	5	0
Time before the β -form is clearly seen, weeks	4	8.5	12.5	35	44	5.5
Visual judgement after 6 weeks	grainy	a bit grainy	not grainy	not grainy	not grainy	grainy

cipes 1 and 2. It took 10 days for recipe 3, 17 days for recipes 4 and 5 and 5 days for recipe 6 which contained 5 % soya 41 instead of LOBRA 40.

The results from the pilot-plant experiments are shown in Table 5. The time before the β -form is clearly seen from the diffraction pattern and a visual judgement after 6 weeks is given.

Discussion

Both lab-scale and pilot-plant experiments show the potential in using diglycerides as a β' -stabilizer. Recipe no. 6, where 5 % soya 41 is added instead of 5 % diglycerides, excludes the possibility that the β' stabilizing effect is due to decreased amount of LOBRA 40. The difference in β' stability between lab-scale and pilot-plant experiments is quite obvious. This difference is partly due to the somewhat higher storage temperature for the lab-scale experiments but also due to the increased stability in the pilot plant margarines caused by the emulsion. The difference is also due to the quick cooling and the stirring in the perfector. In spite of the difference the results point in the same directions. The question is, however,

⁶ L. Hernqvist and K. Larsson, *Fette · Seifen · Anstrichmittel* **84**, 349 [1982].

⁷ K. Larsson, *Acta Chem. Scand.* **20**, 2255 [1966].

how much diglycerides that is needed to get enough stabilization of the β' -form. It seems likely that 1.0 % diglyceride or less is a realistic niveau. The time before the β -form is developed could then be prolonged 2–3 times.

Since the diglycerides are saturated they contribute more than the exchanged LOBRA 40 to the solid phase, but if the amount added is less than 1 % contribution is neglectable.

The diglyceride used in this experiment contains about 26 % triglycerides, which also contributes to the solid phase but without having any delaying effect on the $\beta' \rightarrow \beta$ transition. If diglycerides should be used as a β' -stabilizer it would be better to use a sample that is purer without that much tri-

glycerides. The β' -stabilizing effect is also dependent on chain length of the fatty acids in the diglycerides as well as in which form (rac 1,2, sm-1,2 or 1,3) the diglycerides are⁴. What is of technical relevance is something that must be tested and optimized.

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wind in my heart
take me away

